A Romanian Population Isolate With High Frequency of Vitiligo and Associated Autoimmune Diseases

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Objective: To characterize the epidemiology and genetics of vitiligo and associated autoimmune diseases in a population isolate in Romania in which there is a high frequency of these diseases.

Design: Prospective and retrospective ascertainment of all patients and extended families with these disorders in the study community.

Setting: A geographically isolated community in the mountains of northern Romania.

Patients: Fifty-one affected individuals and their close relatives from 35 nuclear families in an extended kindred that effectively constitutes the entire community population.

Main Outcome Measures: Demographic, phenotypic, and genetic aspects of vitiligo and other autoimmune diseases in the extended kindred.

Results: The frequencies of vitiligo and several other autoimmune diseases, including autoimmune thyroid disease, adult-onset autoimmune diabetes mellitus, and rheumatoid arthritis, are greatly elevated. The age of vitiligo onset in this village is relatively delayed, suggesting that the causes of vitiligo in this community may be somewhat atypical. Genetic segregation analysis is most consistent with a single major locus recessive model, although incomplete penetrance and heritability suggest that other genes and nongenetic factors likely influence occurrence of disease in homozygotes.

Conclusions: The high frequency of vitiligo and other autoimmune diseases in this isolated inbred community and an unusual aspect of the vitiligo phenotype suggest that susceptibility to these disorders in this “special population” may be unusual, likely involving a major genetic component, actual onset of vitiligo in genetically susceptible individuals seems to require exposure to environmental triggers.

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VITILIGO IS AN ACQUIRED noncontagious disorder in which progressive patchy loss of pigmentation of skin, overlying hair, and mucous membranes results from loss of melanocytes from the involved areas. Known for thousands of years because of its visually evident phenotype, vitiligo is the most common pigmentary disease, affecting about 0.38% of whites and occurring with generally similar frequency in other populations worldwide.

Several types of vitiligo are distinguished on clinical grounds (principally, generalized vitiligo, focal vitiligo, and segmental vitiligo). Melanocyte destruction in generalized vitiligo seems to have an autoimmune basis, resulting from a combination of genetic factors and environmental triggers. Generalized vitiligo is epidemiologically associated with other autoimmune diseases, including autoimmune thyroid disease, rheumatoid arthritis, psoriasis, adult-onset type 1 diabetes mellitus, pernicious anemia, systemic lupus erythematosus, and Addison disease in patients with vitiligo and in their close relatives, suggesting that predisposition to this group of autoimmune diseases involves a shared genetic component. Typical generalized vitiligo seems to be inherited as a multifactorial polygenic trait; however, only a few vitiligo susceptibility genes, and no environmental triggers, have been identified with certainty.

A major goal in vitiligo research has been to identify key genes involved in vitiligo susceptibility and to, thereby, identify key disease processes and pathways that might present novel therapeutic tar-
gets. A powerful approach to identifying disease susceptibility genes has been analysis of so-called special populations (ie, groups in which there is high frequency or unusual manifestation of a disease, often associated with genetic isolation). In such situations, disease may result from a restricted number of genes (and potential environmental triggers), the result of a founder effect because of a common ancestor, genetic drift, and inbreeding.13 Herein, we describe characteristics of an isolated inbred community in Romania in which there is a remarkably high frequency of vitiligo and several other autoimmune diseases. All affected individuals descend from relatively few common ancestors, and genetic segregation analysis is most consistent with a single major locus recessive model. This study serves as a starting point for genetic analyses aimed at identifying vitiligo susceptibility genes in this community, genes that likely also play a role in vitiligo susceptibility in the broader white population.

METHODS

STUDY POPULATION

Our study was performed between January 2001 and February 2006, in a community composed of 2 adjoining villages located in a mountainous region of northern Romania. We initially applied an ascertainment questionnaire to the entire population, obtaining demographic data (sex and age) and information about vitiligo (age of onset, extent of disease, mucosal involvement, leukotrichia, presence of Koebner phenomenon, and premature graying of the hair [onset at the age of ≤20 years]), other diseases, and family structure. Questionnaire data were followed up by documentation of clinical histories and by clinical examination by one of us (S.A.B.). Diagnostic criteria for vitiligo were those of the Vitiligo European Task Force,14 classifying vitiligo vulgaris, acrofacial vitiligo, and vitiligo universalis as subtypes of generalized vitiligo, and segmental and local vitiligo as subtypes of localized vitiligo.1 Other forms of leukoderma were excluded. Patients with vitiligo were subjected to successive skin examinations during the study, and all available relatives were examined; ultimately, more than 90% of the village population was examined clinically by one of us (S.A.B.). Another 8 patients with vitiligo were diagnosed by the community physician but died before the present study.

The extent of skin involvement was scored using “Wallace rule of nines.”15 Age of vitiligo onset was defined as the age at which the first white spot was observed, as reported during the clinical interviews of the patients and confirmed by other family members, when possible.

For all patients with vitiligo, we obtained full genealogical data by using the initial questionnaire and successive interviews of the patient and relatives, using data from school, church, and town hall records, and by interviews of the oldest members of the community. We compiled the complete pedigree of affected villagers by tracing back genealogical lineages for each patient for 10 generations (ie, groups in which there is high frequency or unusual manifestation of a disease, often associated with genetic isolation). In such situations, disease may result from a restricted number of genes (and potential environmental triggers), the result of a founder effect because of a common ancestor, genetic drift, and inbreeding. Herein, we describe characteristics of an isolated inbred community in Romania in which there is a remarkably high frequency of vitiligo and several other autoimmune diseases. All affected individuals descend from relatively few common ancestors, and genetic segregation analysis is most consistent with a single major locus recessive model. This study serves as a starting point for genetic analyses aimed at identifying vitiligo susceptibility genes in this community, genes that likely also play a role in vitiligo susceptibility in the broader white population.

STATISTICAL ANALYSES

We used the Fisher exact test to calculate \( P \) values as appropriate. For comparisons with disease frequencies in the general population, we considered the population frequency as a virtually fixed constant, given that it was calculated from a large sample. Consequently, we used the following formula: \( z = (f_1 - f_2)/\sqrt{f_2(1-f_2)/n} \), where \( z \) is the standard normal variate, \( f_1 \) is the frequency in our sample, \( f_2 \) is the frequency in the general population, and \( n \) is the number of individuals in our sample. For statistical analysis of age of onset, we used a procedure (LIFE-TEST) implemented in SAS statistical software (SAS Institute Inc, Cary, North Carolina). Kaplan-Meier estimates were used to construct survival curves (time to event) for the compared groups. Nonparametric comparisons of survival distribution were obtained using the log-rank \( \chi^2 \) and Gehan (Wilcoxon signed rank) tests. We considered \( P < .05 \) (1-sided) as statistically significant.

GENETIC ANALYSES

To formally test different models of genetic inheritance and penetrance, we performed complex segregation analysis of the pedigree as a whole using computer software (Penetrance Estimation option of MENDEL 7.0)15 that implements a generalized linear penetrance model. We used a binomial model with one trial and a logit link function, corresponding to logistic regression over the unknown genotypes at a vitiligo trait locus. Analyses were performed using the entire 624-member pedigree, consisting of 59 affected individuals (35 true probands and 24 affected persons ascertained secondarily), 219 unaffected relatives, and 346 relatives with unknown disease phenotype. Ascertainment correction was applied by conditioning the log likelihood on the proband phenotypes. We examined 3 different major locus models: genotypic (unrestricted), dominant, and recessive, each assuming 2 alleles at the major locus. The fit of the data to the recessive model and the dominant model was compared with the genotypic model using a \( \chi^2 \) test with 1 df, calculated as twice the difference in the natural log likelihoods of the 2 models in each comparison. Analyses were repeated using a grid of different starting values for each variable to rule out convergence to a local maximum. To analyze the heritability \( h^2 \) of age of vitiligo onset, we used computer software (Polygenic and QTL Mapping option of MENDEL 7.0) to partition the total variance into additive genetic and environmental components.

DEMOGRAPHICS

The geographically isolated study community consists of 2 adjacent villages located in a mountainous region of northern Romania. One was founded in 1566 by 2 families and the other in 1603 by 1 family, altogether totaling 23 Romanian white individuals. Since then, the community has grown in continuous isolation, with essentially no historical immigration or emigration. Residents traditionally marry their neighbors, and three-fourths of the population still shares the original 3 family names, with known distant consanguinity in 72% of marriages, although marriages between first cousins is forbidden.

At the most recent census (2004), the community had a population of 1673 individuals. We identified and examined 51 patients with vitiligo (Figure 1), of whom 3
died during the study, yielding a disease prevalence of approximately 2.9% (48/1673). In contrast, among the 2021 inhabitants of the 5 surrounding villages, there were 3 vitiligo cases, yielding a disease frequency of approximately 0.15%. Thus, the frequency of vitiligo in the study community is greatly increased over its frequency in the surrounding villages (P < .001).

Of the 51 patients with vitiligo, 33 were women and 18 were men (Table 1); this seeming female sex bias was not significant (P = .14, 2-tailed). The mean (SD) age was 49.5 (22.8) years (range, 2-83 years). The mean (SD) age of vitiligo onset was 36.5 (19.6) years (median, 37.0 years), vs a mean (SD) of 24.2 (16.2) years (median, 22.0 years) among unselected white probands9 and 21.5 (15.0) years (median, 18.5 years) among probands from multiplex vitiligo cases.10 Survival analysis of age of vitiligo onset (Figure 2) demonstrated that the age of vitiligo onset in the study village is significantly later than among unselected and familial probands with vitiligo (P < .001 for both).

CLINICAL FEATURES

Most patients with vitiligo had generalized (82%) or active (71%) forms of the disease (Table 1). Of the 42 patients with generalized vitiligo, 31 (74%) had the vulgaris subtype, 10 (24%) had the acrofacial subtype, and 1 (2%) had the universalis subtype. Among the 9 patients with localized vitiligo, 8 (89%) had the focal sub-

Figure 1. Pedigree of the extended vitiligo kindred in the Romanian study community. In some instances, consanguinity was reported, but the precise relationship was not known. Circles indicate females; squares, males; shaded symbols, those with vitiligo; symbols with a slash mark, deceased individuals; green bars, those affected by autoimmune thyroid disease; orange bars, those affected by rheumatoid arthritis; blue bars, those affected by type 1 diabetes mellitus; brown bars, those affected by psoriasis; red bars, those affected by systemic lupus erythematosus; and yellow bars, those affected by autoimmune-type chronic active hepatitis.
type and 1 (11%) had segmental vitiligo. As shown in Table 1, among the total 51 patients with vitiligo, the clinical findings of leukotrichia, mucosal involvement, Koebner phenomenon, and premature graying of hair were relatively uncommon, even among patients with generalized vitiligo. Nevertheless, leukotrichia, mucosal involvement, and Koebner phenomenon virtually only occurred in patients with generalized vitiligo and active disease and, thus, constitute indicators for this form of the disorder.

We estimated fractional skin surface involvement in quartiles: less than 25%, 25% to 50%, 51% to 75%, and greater than 75%. As shown in Table 1, 47% of patients had generalized vitiligo with less than 25% skin surface involvement. Fractional skin surface involvement was directly correlated with disease duration in most patients. Of the 34 patients with disease duration of 15 years or less, 28 (82%) had less than 25% skin surface involvement; in contrast, of the 17 patients with disease duration of longer than 15 years, only 5 (29%) had less than 25% skin surface involvement ($P < .001$).

ASSOCIATED AUTOIMMUNE DISEASES

Previous studies have shown epidemiological association of generalized vitiligo with a number of other autoimmune diseases, including autoimmune thyroid disease, rheumatoid arthritis, adult-onset type 1 diabetes mellitus, psoriasis, pernicious anemia, systemic lupus erythematosus, and Addison disease. In a preliminary analysis of a subset of the study community, we found elevated frequencies of autoimmune thyroid disease, rheumatoid arthritis, and adult-onset type 1 diabetes mellitus, in patients with vitiligo and in their first-degree relatives.

As shown in Figure 1 and Table 2, our findings in the entire community were generally similar. Of the 51 total patients with vitiligo, 22 (43%) had 1 or more of the other autoimmune diseases that are epidemiologically associated with vitiligo, not significantly different ($P = .06$) than the frequency of other autoimmune diseases among typical Romanian patients with generalized vitiligo. Almost all (21 of 22 [95%]) of the patients with vitiligo and other autoimmune diseases had generalized vitiligo. Of the 22 patients with vitiligo who had multiple autoimmune diseases, 10 had autoimmune thyroid disease, 5 had autoimmune thyroid disease and rheumatoid arthritis, 5 had type 1 diabetes mellitus, 1 had rheumatoid arthritis, and 1 had

<table>
<thead>
<tr>
<th>Feature</th>
<th>Total b</th>
<th>Patients With Generalized Vitiligo</th>
<th>Patients With Nongeneralized Vitiligo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>33 (65)</td>
<td>26 (51)</td>
<td>7 (14)</td>
</tr>
<tr>
<td>Male</td>
<td>18 (35)</td>
<td>16 (31)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Disease activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>36 (71)</td>
<td>35 (69)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Stable</td>
<td>15 (29)</td>
<td>7 (14)</td>
<td>8 (16)</td>
</tr>
<tr>
<td>Other clinical features recorded</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Koebner phenomenon</td>
<td>20 (39)</td>
<td>19 (37)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Leukotrichia</td>
<td>10 (20)</td>
<td>10 (20)</td>
<td>0</td>
</tr>
<tr>
<td>Premature graying of the hair d</td>
<td>13 (25)</td>
<td>11 (22)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Mucosal involvement</td>
<td>6 (12)</td>
<td>6 (12)</td>
<td>0</td>
</tr>
<tr>
<td>% of body surface involved</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>33 (65)</td>
<td>24 (47)</td>
<td>9 (18)</td>
</tr>
<tr>
<td>25-50</td>
<td>4 (8)</td>
<td>4 (8)</td>
<td>0</td>
</tr>
<tr>
<td>51-75</td>
<td>13 (25)</td>
<td>13 (25)</td>
<td>0</td>
</tr>
<tr>
<td>&gt;75</td>
<td>1 (2)</td>
<td>1 (2)</td>
<td>0</td>
</tr>
</tbody>
</table>

*Data are given as number (percentage) of the 51 patients. Of the patients, 42 (82%) were in the generalized vitiligo group and 9 (18%) were in the nongeneralized vitiligo group.

*These percentages may not be the exact sum of the percentages given in the patients with generalized vitiligo and the patients with nongeneralized vitiligo groups because of rounding.

*Refers to expansion of existing lesions or the appearance of new lesions from 2001 to 2006.

*Defined as starting at the age of 20 years or younger.
autoimmune thyroid disease, rheumatoid arthritis, and type 1 diabetes mellitus.

Of the 51 patients with vitiligo, 31% had autoimmune thyroid disease. This is significantly more than its approximate 1.90% frequency in the general US population17 and its 17.0% frequency among unselected white probands with vitiligo (P < .001),9 and similar (P = .15) to its 22.6% frequency among white probands with familial vitiligo.10

Of the 51 patients with vitiligo, 7 (14%) had rheumatoid arthritis. This is significantly more than its approximate 0.86% frequency in the general US population (P < .001)17 and its 0.67% frequency among unselected white probands with vitiligo,9 and more than its 3.8% frequency among white probands with familial vitiligo (P = .02).10

Of the 51 patients with vitiligo, 6 (12%) had type 1 diabetes mellitus, 3 (6%) had typical juvenile diabetes mellitus, and 3 (6%) had adult-onset autoimmune diabetes mellitus (latent autoimmune diabetes of adults). These are both far more frequent than the estimated population frequencies of juvenile diabetes mellitus (0.48%17) (P < .001) and of latent autoimmune diabetes of adults (0.59%18,19) (P < .001). The overall 11.8% frequency of juvenile diabetes mellitus among the 51 patients with vitiligo was significantly greater (P = .003) than its 0.48% frequency among unselected white probands with vitiligo,9 although the 6% frequency of latent autoimmune diabetes of adults in the 51 patients with vitiligo (P = .62) was similar to that among white probands with familial vitiligo (3.8%).10

These same autoimmune diseases also occurred at increased frequencies among the 172 parents and siblings of the 35 true probands. Altogether, 15 of these first-degree relatives had vitiligo, significantly more than the 2.9% frequency of vitiligo in the community (P < .001), and far more (P < .001) than the 0.15% frequency of vitiligo in the 5 surrounding villages and its 0.38% frequency (P < .001) among whites from Bornholm, Denmark.3 Likewise, as shown in Table 2, the frequencies of other vitiligo-associated autoimmune diseases were also significantly elevated among first-degree relatives of probands with vitiligo in the study community compared with their frequencies in the general population: autoimmune thyroid disease, 12%; rheumatoid arthritis, 3%; and type 1 diabetes mellitus, 3%. Even among vitiligo probands’ first-degree relatives who did not themselves have vitiligo, the frequencies of autoimmune thyroid disease (9.5%, P < .001) and type 1 diabetes mellitus (2.54%, P < .001) were significantly elevated, and that of rheumatoid arthritis was perhaps marginally elevated (1.91%, P = .07), suggesting that the study community segregates genetic risk factors that contribute to an autoimmune diathesis that includes vitiligo, autoimmune thyroid disease, rheumatoid arthritis, and autoimmune diabetes mellitus.

**GENETICS AND SEGREGATION ANALYSIS**

The 51 patients with vitiligo derived from 44 nuclear families. Forty-nine patients (96%) could be linked in a single extended kindred (Figure 1), although 2 patients were only indirectly related to other cases via their spouses. In addition, another 8 patients with vitiligo diagnosed by the community physician but deceased before the present study were included in the pedigree.

Most marriages show distant consanguinity, typically involving second to fifth cousins. In 35 of the 44 nuclear families (79.5%), both parents of an affected individual were themselves unaffected, although in 8 nuclear families (18.2%), 1 parent was affected. There was 1 mating between 2 affected parents, 3 of whose 6 offspring were affected with vitiligo. The 35 true probands had 6 affected and 55 unaffected siblings, plus another 41 siblings for whom we lacked phenotype data, most of whom are deceased. Among the probands’ 61 siblings for whom we have phenotypic data, the frequency of vitiligo is, thus, 9.8%, significantly greater (P = .01) than the 2.9% frequency of vitiligo in this community, but not significantly different (P = .17) than the 6.1% frequency of vitiligo in the 5 surrounding villages and its 0.38% frequency (P < .001) among whites from Bornholm, Denmark.3 Likewise, as shown in Table 2, the frequencies of other vitiligo-associated autoimmune diseases were also significantly elevated among first-degree relatives of probands with vitiligo in the study community compared with their frequencies in the general population: autoimmune thyroid disease, 12%; rheumatoid arthritis, 3%; and type 1 diabetes mellitus, 3%. Even among vitiligo probands’ first-degree relatives who did not themselves have vitiligo, the frequencies of autoimmune thyroid disease (9.5%, P < .001) and type 1 diabetes mellitus (2.54%, P < .001) were significantly elevated, and that of rheumatoid arthritis was perhaps marginally elevated (1.91%, P = .07), suggesting that the study community segregates genetic risk factors that contribute to an autoimmune diathesis that includes vitiligo, autoimmune thyroid disease, rheumatoid arthritis, and autoimmune diabetes mellitus.

**Table 2. Autoimmune Diseases in the 51 Patients With Vitiligo and in Their First-Degree Relatives**

<table>
<thead>
<tr>
<th>Autoimmune Disease</th>
<th>Patients With Vitiligo (n=51)</th>
<th>Probands’ First-Degree Relatives (n=172)</th>
<th>Population Frequency, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitiligo</td>
<td>51 (100)</td>
<td>15 (9)</td>
<td>0.38&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Autoimmune thyroid disease</td>
<td>16 (31)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21 (12)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.90&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hashimoto thyroiditis</td>
<td>8 (16)</td>
<td>5 (3)</td>
<td>NA</td>
</tr>
<tr>
<td>Autoimmune thyroid disease with goiter</td>
<td>3 (6)</td>
<td>7 (4)</td>
<td>NA</td>
</tr>
<tr>
<td>Subclinical autoimmune thyroid disease</td>
<td>5 (10)</td>
<td>6 (3)</td>
<td>NA</td>
</tr>
<tr>
<td>Graves disease</td>
<td>0</td>
<td>2 (2)</td>
<td>NA</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>7 (14)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5 (3)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.86&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Autoimmune diabetes mellitus</td>
<td>6 (12)</td>
<td>5 (3)</td>
<td>NA</td>
</tr>
<tr>
<td>Juvenile-onset type 1 diabetes mellitus</td>
<td>3 (6)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5 (3)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.48&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Adult-onset autoimmune diabetes mellitus</td>
<td>3 (6)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
<td>0.59&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Abbreviation: NA, data not available.

<sup>a</sup>Indicated P values are for comparison of observed frequency with population frequency of each disease. Data in parentheses are percentages.
<sup>b</sup>P < .001.
<sup>c</sup>Data derived from Howitz et al.<sup>3</sup>
<sup>d</sup>Data derived from Jacobson et al.<sup>17</sup> and the US census.
<sup>e</sup>P = .02.
<sup>f</sup>Data derived from Pozzilli and DiMario<sup>18</sup> and Koopman et al.<sup>19</sup>
ligo among siblings of unselected white probands with vitiligo.

We performed formal segregation analysis of the vitiligo phenotype using the entire 624-member pedigree. As shown in Table 3, the best fit to the data was given by a single major locus recessive model with penetrance of 0.30 for affected homozygotes and disease allele frequency of 0.34. These penetrance and allele frequency estimates for the recessive model agree remarkably well with the estimates from an unrestricted model that does not assume any particular mode of inheritance. The prevalence estimates for different models also agree well with the observed prevalence. By comparison, a single locus–dominant model gave a poorer fit to the data compared with the unrestricted model, although it was not possible to reject a dominant model at the criterion significance level of $P < .05$. Thus, it seems that a single recessive locus may be necessary, but not sufficient, for the occurrence of vitiligo in the study community.

We performed formal analysis of the heritability ($h^2$) of age of vitiligo onset in patients, partitioning the total variance into additive genetic and environmental components. We found no evidence that genetic factors contribute to the variance of age of vitiligo onset ($h^2=0$); instead, age-of-onset differences seem to be largely or entirely explained by environmental factors.

Table 3. Genetic Segregation Analysis of Vitiligo in the Study Community

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unrestricted</th>
<th>Recessive</th>
<th>Dominant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penetrance 1-1</td>
<td>b</td>
<td>b</td>
<td>0.03</td>
</tr>
<tr>
<td>Penetrance 1-2</td>
<td>b</td>
<td>b</td>
<td>0.24</td>
</tr>
<tr>
<td>Penetrance 2-2</td>
<td>0.30</td>
<td>0.30</td>
<td>0.24</td>
</tr>
<tr>
<td>Frequency 1</td>
<td>0.66</td>
<td>0.66</td>
<td>0.93</td>
</tr>
<tr>
<td>Frequency 2</td>
<td>0.34</td>
<td>0.34</td>
<td>0.07</td>
</tr>
<tr>
<td>Prevalence</td>
<td>0.04</td>
<td>0.04</td>
<td>0.03</td>
</tr>
<tr>
<td>Logarithm of the likelihood</td>
<td>−75.61</td>
<td>−75.61</td>
<td>−77.30</td>
</tr>
<tr>
<td>$P$ value</td>
<td>NA</td>
<td>&gt; .99</td>
<td>.07</td>
</tr>
</tbody>
</table>

Abbreviation: NA, data not applicable.

COMMENT

We have investigated a geographically isolated inbred community in a mountainous region of northern Romania in which there are greatly elevated frequencies of vitiligo and several other autoimmune diseases that are epidemiologically associated with vitiligo. The 2.9% frequency of vitiligo in the study community is 19.3 times its 0.15% frequency in the 5 surrounding villages, 7.5 times that among whites on the island of Bornholm, 1.5 times that among individuals in CALCUTTA, India, and 22.5 times that among Han Chinese in Shaanxi Province, China, the only other populations for which empirically determined prevalence estimates have been published.

The clinical characteristics of vitiligo in the Romanian study community are generally similar to those reported elsewhere. The most frequent pattern was generalized vitiligo involving less than 25% of the skin surface. As has been observed in other populations, the frequencies of several other autoimmune diseases, including autoimmune thyroid disease, adult-onset type 1 diabetes mellitus, and rheumatoid arthritis, are elevated in patients with vitiligo and in their first-degree relatives, most likely because of a gene or genes that predispose to this group of autoimmune diseases.

Interestingly, the mean age of vitiligo onset (36.5 years) was significantly later than among unselected white patients with vitiligo (24.2 years) and patients from white families with multiple cases of vitiligo (21.5 years). This unusual aspect of vitiligo in the study community may reflect unusual aspects of its causation in this isolated community, which has been a virtually closed population since its founding 4 centuries ago, because of geographical isolation, and, thus, has had prevalent known and occult inbreeding. Such “special populations” offer powerful opportunities to map and identify disease genes, particularly recessive genes, because overall causal genetic heterogeneity in such populations has been reduced by founder effect, inbreeding, and genetic drift. Amplification of a specific causal genetic variant shared by affected members of a special population may, thus, result in a disease phenotype that is somewhat atypical compared with patients from outbred populations, in which genetic and environmental heterogeneity may be much greater.

Typically, family aggregation of vitiligo cases occurs in a nonmendelian pattern suggestive of polygenic multifactorial inheritance. Genetic segregation analyses indicate that vitiligo typically involves multiple major loci contributing to disease risk in a complex interactive manner. In contrast, genetic segregation analysis of the present study community supports a single major recessive locus with incomplete penetrance, consistent with reduced causal genetic heterogeneity in this isolated and relatively inbred population, although this analysis does not exclude more complex causation. The estimated penetrance of 0.30 suggests that additional genetic and/or nongenetic factors influence occurrence of vitiligo even in this population. Indeed, heritability analysis indicated that age of vitiligo onset in the study community is determined almost completely by nongenetic environmental factors. Thus, whereas a major gene or genes seems to govern susceptibility to vitiligo, actual onset of disease seems to depend on exposure of genetically susceptible individuals to environmental triggers.

This Romanian community may present a facile opportunity to map and ultimately identify a recessive gene that confers high risk of vitiligo and associated autoimmune diseases. Even 4 centuries after the community’s founding, there would still be almost complete linkage disequilibrium surrounding a causal founder genetic variant to a distance of at least 100 kilobase, facilitating identification of the causal gene by methods that assume identity by descent from the common founder ancestor. While this gene variant is of particular importance in this iso-
lated special population, it likely is also involved in disease susceptibility in the broader white population and, thus, is of broader importance.

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Author Contributions: Study concept and design: Birlea and Spritz. Acquisition of data: Birlea and Spritz. Analysis and interpretation of data: Birlea, Fain, and Spritz. Drafting of the manuscript: Birlea, Fain, and Spritz. Critical revision of the manuscript for important intellectual content: Fain and Spritz. Statistical analysis: Birlea and Fain. Obtained funding: Spritz. Administrative, technical, and material support: Birlea. Study supervision: Fain and Spritz.

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