A FAMILY STUDY OF LYMPHEDEMA OF THE LEG IN A LYMPHATIC FILARIASIS–ENDEMIC AREA

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Abstract. The risk of filarial lymphedema may not be equivalent for all members of filaria-exposed populations. While evidence for a genetic factor that influences acquisition of infection has been growing, very little work has addressed whether there is a genetic basis to the development of disease due to lymphatic filariasis. We designed a family study of lymphedema in a rural community in Haiti to assess disease aggregation. Three hundred sixty-eight female patients sixteen years of age or older were enrolled at a lymphedema treatment clinic between June 1995 and December 1999. After applying additional eligibility criteria, 172 probands were enrolled into the family study for detailed pedigree collection between September 1998 and December 1999. Fifty-three lymphedema cases were identified among the probands’ parents, full-siblings, children, half-siblings, and mating partners of the parents. Twelve of the 53 cases were among males. The proportion of cases occurring in a biologic parent of the proband was higher than in unrelated individuals married into the proband’s family (P = 0.0010). This is the first large family study based on pedigrees to assess the familial aggregation of lymphedema due to filariasis. This family study will be useful to investigate the role of genes and environment in the development of filarial-related lymphedema.

INTRODUCTION

Although the presence of adult filarial worms of Wuchereria bancrofti may lead to the development of lymphangiectasia, most persons with long-term infections are clinically asymptomatic. Infection leads to lymphedema, a build up of fluid due to impaired function of the lymph vessels, in only a small proportion of persons, even in areas of intense transmission. Some of these lymphedema patients progress to elephantiasis as a consequence of recurrent bacterial infections. Lymphedema is present more often in women than in men, but regardless of gender, affected persons experience stigmatization, economic impairment from decreased mobility, and painful acute attacks of adenolymphangitis.

Several observations are consistent with the hypothesis that a small proportion of filarial-exposed populations may be genetically predisposed to lymphedema development, but these observations have been interpreted from an immunologic perspective. First, in some endemic settings, persons with lymphedema are significantly more likely to be negative for microfilaria and circulating filarial antigen than age-matched controls, suggesting a possible relationship between protective immunity and disease development. Second, lymphedema patients have antifilarial immune responses that are significantly greater than those of antigen-negative persons without overt disease. Furthermore, both filarial infection and antifilarial immunity appear to cluster by family. One study specifically addressed familial aggregation of infection markers. Terheli and others found that microfilarial status aggregated within families and was not due to shared environment. When filarial infection status was assessed using antifilarial IgG4 levels as a proxy for infection, it was also found to cluster within families. It could not be determined from this study to what degree the clustered responses were the product of shared genes or environmental factors that could influence host-vector contact such as proximity to vector habitat. The hypothesis that genes control infection status was strengthened in an independent study that found an association between candidate gene variants and filarial infection.

While the evidence for a genetic component that influences acquisition of infection has been growing, very little work has assessed the influence of genetics on the development of disease due to lymphatic filariasis. In two studies, specific HLA antigens were associated with elephantiasis, supporting the concept that there is a genetic component that influences lymphedema development, but no such relationship was observed in a previous study.

We designed and initiated a family study of filarial lymphedema in Haiti with the primary goal of assessing disease aggregation and determining whether the frequency of lymphedema was higher in relatives of lymphedema patients than in the general population. A secondary goal of the investigation was to explore potential risk factors affecting pathogenesis of lymphedema. We focused specifically on factors that were believed to increase exposure to filarial worms and/or secondary bacterial infections that may also be related to disease. The original study population and methods being used to address these objectives are described here.

MATERIALS AND METHODS

Study site. The lymphedema treatment clinic at Hôpital Ste. Croix in Leogane, Haiti, a coastal community 30 km west of Port-au-Prince, was established as part of a research study to investigate the feasibility of low cost approaches to the treatment of lymphedema. The initial 30 patients with lymphedema of the leg were invited to enroll at the clinic in June 1995. Recruitment was stimulated by word of mouth and clinic outreach efforts. Patients were educated about limb care, skin hygiene, exercises, proper use of compressive bandages, and management of acute attacks of adenolymphangitis as a means of treating lymphedema due to filariasis. Antibiotic therapy was provided when clinic staff felt it was indicated. Regular follow-up of patients provided an opportunity to measure limb circumference and volume and to assess filarial infection status.

Study population and pedigree collection. Female patients enrolled at the lymphedema treatment clinic were recruited as probands for the family study. We focused on female pa-
tients because lymphedema in Haiti is predominantly a disease of women. Three hundred seventy-five of 463 clinic lymphedema cases were female. We designated the first of related lymphedema cases to enroll at a clinic as the proband to minimize overestimation of families with multiple lymphedema cases. Relatives who enrolled after the proband were typically found to be first-degree relatives who were then included for pedigree analyses. Probands also had to be accessible for interview and to have enrolled at the clinic between June 1995 and December 1999. The clinic registry contained names of 368 women ≥ 16 years of age. Eight of these women were identified as the second person from a family to enroll at the clinic. Female relatives who enrolled after the proband’s enrollment date were excluded from being a proband.

All probands were interviewed at their homes between September 1998 and December 1999 for pedigrees and addresses for living relatives. If a proband was not present at the time of the interview, a relative (≥ 16 years of age) who lived with the proband at the time of the interview was permitted to give information on her behalf. The pedigree collection was done in Creole by trained interviewers. Participants gave oral consent to pedigree collection. Consent forms were translated into Creole, read, and explained to each study subject. The study protocol was reviewed and approved by the Institutional Review Board of the Centers for Disease Control and Prevention and by the Ethics Committee of Hôpital Ste. Croix.

Interviewers were taught to adjust the complexity of questions and to communicate pedigree concepts. Particular attention was given to differentiating between biologic and adopted relatives. Interviewers used a set of questions as a guide to obtain a pedigree that focused on parents, children, full siblings, and half-siblings of the proband. Each interviewer was required to participate in practice interviews until no mistakes were made on two consecutive interviews. Pedigrees from these interviews were compared with those prepared earlier by two of the authors (K.T.C and J.L.C.). When pedigree collection was done on study probands, clinic staff confirmed any reports of relatives with lymphedema. Only the proband providing pedigree information had to be ≥ 16 years old. There was no age restriction for family members to be included in the pedigree drawing or subsequent analysis. A subset of probands and selected controls were administered an additional questionnaire assessing behavioral risk factors for lymphedema, including personal hygiene and mosquito avoidance practices (to be reported elsewhere).

RESULTS

Between November 1997 and February 1998 (Figure 1), clinic staff believed that the clinic had enrolled more than 80% of male and female lymphedema cases in the area served by the hospital (n = 260). However, additional cases enrolled after March 1998 when a radio campaign for lymphatic filariasis education was started. During the time period following the campaign, March 1998 to July 1999, 203 male and female lymphedema patients enrolled at the clinic. This suggested that the clinic registry had ascertained approximately 50% of the lymphedema cases in the area. Individuals who enrolled after December 1997 tended to live further away than individuals who enrolled earlier.

Of the 368 women in the clinic registry, 92 women were geographically inaccessible and three women had died before interviewing began. Seventy-nine women had unknown addresses because clinic staff lacked the familiarity with these patients that was needed to locate them. Eight individuals were the second member from a family to enroll at the clinic. The remaining 186 lymphedema patients were approached for interview. Repeated contact attempts failed for four women. Five patients were excluded due to interviewer comments indicating an incomplete pedigree. Five other women were mentally incapable of providing pedigree information. The remaining 172 cases had completed pedigrees (Figure 1). None of the probands refused to be interviewed.

Probands ranged from 16 to 81 years of age (Figure 2). The mean proband age was 40.9 years and the median age was 41 years. Interviews of lymphedema patients revealed that overall family or pedigree size, which included first-degree relatives, half-siblings, and children of half-siblings of the proband, was quite large. The size of study pedigrees ranged from 6 to 40 persons with a mean family size of 15.3 persons. Fifty percent of families had between 11 and 18 family members (Figure 3). More than 2,000 individuals were included in the study pedigrees (sample pedigree in Figure 4). The mean numbers of full siblings and half-siblings in each family were 8.7 and 3.0, respectively.
Clinic records indicated that three of 53 lymphedema cases identified in addition to the probands were already enrolled. Twelve of the 53 cases were males. The largest number of additional lymphedema cases was reported among parents of the proband (22 of 53 = 42%) (Table 1). Additional lymphedema was reported only among full-siblings (13 of 53 = 25%), children (9 of 53 = 17%), half-siblings (7 of 53 = 13%), and mating partners of the parents (non-biologic relative of the proband) (2 of 53 = 4%).

The majority of multiple cases of lymphedema in relatives of the proband was 2.4% (53 of 2,235). The prevalence of lymphedema was highest in parents (6.4%) and full siblings (2.1%) of the proband. The observed decrease in prevalence with biologic distance, e.g., full sibling, half sibling, and unrelated mating partner of parent, was not statistically significant ($P = 0.31$, by two-sided exact trend test). When unrelated individuals were contrasted with each other type of relative, only the comparison of prevalence between unrelated individuals and parents of the proband was significantly different ($P = 0.0010$). When family sizes were collapsed into categories of 6–10, 11–15, 16–20, 21–25, and $\geq 26$ individuals per family, multiple cases of disease were more frequent in families consisting of 11–15 and 16–20 person categories (Table 2); however, these were the most commonly observed family sizes.

**DISCUSSION**

This is the first extensive family study to collect pedigrees to assess genetic and non-genetic factors associated with lymphedema in a Haitian community and to address familial aggregation of filarial-related lymphedema. Earlier work could not be used to assess filarial disease aggregation in families. Several studies focused on correlating infection and immune status between mothers and their children, but were too small to address aggregation of disease specifically.5–10

The single published study reporting on familial clustering did not include an assessment of disease status, instead focusing on infection only.11 The sole candidate gene study suggesting some association between infection and gene variants is promising, but needs to be replicated in other settings.12

These data suggest the possible presence of a crude trend between degree of relatedness to the proband and disease development. Although it was not statistically significant, all relative-types of the proband appeared to have higher proportions of disease than found in unrelated spouses. Only the prevalence of disease among parents of the proband was significantly higher than that in unrelated individuals.

The difference in disease proportions between parents of the proband and unrelated spouses of the parent is suggestive and may be attributable to differences in genetic distance from the proband, assuming that age distribution and environmental factors that influence exposure to the vector were similar for the members of the oldest generation. If this result extends to results from additional studies that control for environmental factors, then this would support the hypothesis that lymphedema is, at least in part, genetically determined. We are currently assessing environmental survey data to determine the validity of these assumptions. If the crude trend in lymphedema prevalence that we observed for relatives versus unrelated individuals is found to be significant, it would be consistent with the hypothesis that increased relatedness to a proband with lymphedema is associated with increased probability of lymphedema development.

The identification of additional lymphedema patients in the proband families did not appear to be dependent on family size. The majority of multiple cases of lymphedema occurred in large (28 of 53 = 53% from families with 11–20 persons) families, which were the most frequently occurring, but certainly not the largest families. The proportion of multiple affected families remained steady as family size increased.

The feasibility of assessing familial aggregation and identifying high-risk families hinged upon improving the probability of identifying families with potentially increased disease risk, the quantity and content of interview information, and the relevance of the study objective to the Haitian community. The method for selecting families was chosen based on the rarity of the disease outcome. The recruitment of affected probands and their respective family members through a clinic setting provided an efficient means of detecting multiple cases of lymphedema in a family. To avoid ascertainment bias, a random sample of families in the area that the clinic served was considered in the early planning stages of the current study. Although the alternate design would have produced an additional set of lymphedema prevalence estimates, we determined that it was impractical. Due to the low prevalence of lymphedema in this area of Haiti (1–2%)10 (Lammie PJ, unpublished data), it would have required a significant investment of resources to examine enough families to obtain a sufficient number of lymphedema cases to allow for familial aggregation assessment.

Assuming that the clinic patients would be the most efficient way to recruit families with disease, we considered how to handle the observation that clinic coverage of affected women was incomplete. Enrollment patterns indicate that the

**FIGURE 3.** Distribution of family sizes (n = 172) for families where pedigrees were collected. Pedigrees included parents, children, full siblings, and half-siblings of the proband. There were no age limits placed on family members.

**FIGURE 4.** Sample pedigree. ---- = informal relationship; ---/--- = separation of informal relationship; ♂ = proband; ○ = female; □ = male; □ = deceased male. Filled symbols represent persons with lymphedema.
for their demographic information. Obviously, the present studies in this population will be able to focus on informative it is not possible to determine this from our study. Future

Number of persons other than proband with disease as a function of
edema than unrelated partners of the probands

explan why more parents were reported as having lymph-

logic versus non-biologic relatives of the proband. This may
may be a differential recall of information pertaining to bio-

there also

data errors. When biologic parental lines were ex-

one way to determine how well we are identifying

lymphedema in families is through a separate assessment of

clinic outreach abilities that would require a sample obtained independently of the registry.

Interviewees were willing to provide family disease history and relations, but there was some difficulty with demographic details and delineating relationships. As the study progressed, it was apparent that membership in an extended family appears to be commonplace in this setting and little overt distinction is made between half- and full-siblings. While interviewers made a point to clarify the biologic relationship of both parents to a child, misunderstandings may have introduced data errors. When biologic parental lines were explored through detailed interviews, half-siblings were common, a point missed in previous family surveys in this setting. Respondents tended to have difficulty recalling ages of family members, which resulted in missing data. There also may be a differential recall of information pertaining to biologic versus non-biologic relatives of the proband. This may explain why more parents were reported as having lymphedema than unrelated partners of the probands’ parents, but it is not possible to determine this from our study. Future studies in this population will be able to focus on informative family branches and those individuals will be directly queried for their demographic information. Obviously, the present

<table>
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<th>Relation to proband</th>
<th>Number of cases</th>
<th>Male cases</th>
<th>Female cases</th>
<th>Number in pedigrees</th>
<th>Proportion of total (53) additional cases</th>
<th>Prevalence in pedigrees</th>
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<tr>
<td>Child</td>
<td>9</td>
<td>2</td>
<td>7</td>
<td>522</td>
<td>0.17</td>
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<tr>
<td>Half-sibling</td>
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<td>3</td>
<td>5</td>
<td>513</td>
<td>0.15</td>
<td>0.016</td>
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<tr>
<td>Parent</td>
<td>22</td>
<td>4</td>
<td>18</td>
<td>342</td>
<td>0.42</td>
<td>0.064</td>
</tr>
<tr>
<td>Full-sibling</td>
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<td>9</td>
<td>627</td>
<td>0.23</td>
<td>0.019</td>
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<td>Mating partner of parents</td>
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<td>2</td>
<td>231</td>
<td>0.04</td>
<td>0.009</td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>12</td>
<td>41</td>
<td>2235</td>
<td>1.00</td>
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Family size

<table>
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<th>Family size</th>
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<th>Number of families</th>
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<tr>
<td></td>
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</tr>
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<td>11–15</td>
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<td>3</td>
</tr>
<tr>
<td>≥26</td>
<td>7</td>
<td>2</td>
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analysis is based on the assumption that the data collected have a reasonable level of reliability.

Motivation for participation at the clinic appeared to have been quite high; 100% of the patients approached from the lymphedema treatment clinic agreed to participate in pedigree interviews, reflecting the community’s concern about filarial disease. In a survey of knowledge, attitudes, and perceptions of illnesses of this community, lymphedema was ranked by community members as the second most serious illness, exceeded only by acquired immunodeficiency syndrome. For persons affected with lymphedema, their initial clinic visit was the first time that they had been told that there was treatment for their disease. The offering of this hope to patients played a role in compliance as the debilitating and progressive nature of the disease affects potential earning ability and can stigmatize affected persons.

These early descriptions of Haitian family size and distribution of lymphedema in a lymphatic filariasis—endemic area provide a snapshot of lymphedema clustering. One observation suggests disease was present more often in the proband’s biologic parents than unrelated individuals partnered with the proband’s parent. This is suggestive of a shared exposure, possibly genetic, but not ruling out an environmental one. It is possible that more closely related individuals with lymphedema share genes contributing to disease development more often than unrelated individuals. These genes could be involved in regulation of immune responses, determining susceptibility to secondary bacterial infections, or influencing other factors that affect disease progression. Although our data are suggestive of a role for genetic factors in lymphedema development, additional studies will be needed to determine which genes may be involved. With the present study, we have the unique opportunity to explore the extent of genetic influence in one of the few family studies performed for filariasis. The data being collected, including pedigree and disease status, will be useful for planning additional studies of filarial disease and the ability of the lymphedema clinic to serve the population. Improving and different analytical approaches are taken, a better picture of the role of genetics and lymphatic filariasis will develop.

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