Human Lymphatic Filariasis: Genetic Polymorphism of Endothelin-1 and Tumor Necrosis Factor Receptor II Correlates With Development of Chronic Disease

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Background. Hydrocele and elephantiasis are 2 clinically very diverse and often mutually exclusive chronic manifestations of human bancroftian filariasis. Plasma levels of endothelin-1 (ET-1), a major angiogenic factor, and tumor necrosis factor receptors (TNFRs) that regulate host inflammation have been associated with development of chronic filariasis, although their genetic basis are not known.

Methods. We studied polymorphisms of ET-1 (Ala288Ser) and TNFR-II (Met196Arg) genes by means of the polymerase chain reaction confronting 2 pairs primers method and restriction fragment length polymorphism, respectively. Plasma ET-1 level was measured by enzyme-linked immunosorbent assay.

Results. Met196Arg genotype frequency of TNFR-II polymorphism was significantly greater in hydrocele patients, compared with elephantiasis patients (OR, 4.34 [95% CI, 2.04–9.20]). Conversely, a significantly high prevalence of the Ala288Ser mutation of ET-1 was observed in elephantiasis patients, compared with hydrocele cases (OR, 2.15 [95% CI, 1.13–4.10]). Decreased plasma ET-1 levels associated significantly with Ala288Ser mutation in the study population. A combined analysis indicated a 23-fold higher risk for developing elephantiasis in individuals with TNFR-II (Met196Met) and ET-1 mutants (Ala288Ser + Ser288Ser).

Conclusions. ET-1 (Ala288Ser) and TNFR-II (Met196Arg) polymorphisms are associated with development of one or the other form of chronic disease in bancroftian filariasis.
limited. Chitotriosidase I and mannose-binding lectin 2 (MBL2) gene polymorphisms were shown to be associated with increased susceptibility to filarial infections in one study [8], whereas this could not be confirmed in another geographical area, viz. Papua New Guinea [9]. A recent report revealed a significant association between MBL genotypes and the presence of circulating filarial antigens in Africa [10]. Lack of correlation of Toll-like receptor 4 polymorphism with infection status or disease phenotype has been reported in a Melanesian population [9]. A significant association between vascular endothelial growth factor A (VEGF-A) gene promoter polymorphism and increased susceptibility for development of hydrocele in patients infected with W. bancrofti was demonstrated recently [11].

Endothelin-1 (ET-1) plays a key role in inflammation, particularly in connective tissue fibrosis. The endothelin system has a role in physiological vasoconstriction, as well as in the pathophysiology of systemic endothelial activation [12]. Increased ET-1 levels have been associated with vascular hypertrophy and fibrosis [13]. Because ET-1 promotes extravasations of fluid and plasma proteins into the surrounding tissues, it has been proposed that progression of infection to chronic disease manifestations in filariasis could be due to overexpression of lymphangiogenic factors, such as VEGF and endothelin [11, 14]. The human ET-1 gene in chromosome 6 spans approximately 5500 base pairs with 5 exons and 4 introns [15]. The presence of a novel polymorphism, the G/T with an amino acid substitution Ala to Ser at the codon 288 position, was reported in a Japanese population [16]. The functional relevance of this common polymorphism (Ala288Ser), however, is unclear.

We have previously reported a role for the inflammatory cytokine tumor necrosis factor α (TNF-α) in acute filarial disease [17]. The biological activities of TNF are mediated by 2 structurally related but functionally distinct receptors, TNFR-I and TNFR-II, belonging to the TNFR gene family. Whereas TNFR-I mediates inflammatory signaling, TNFR-II often functions as a TNF-α antagonist [18]. A significant association of plasma levels of TNF-I and TNFR-II with chronic disease in filariasis was revealed by us a few years ago—lymphedema and hydrocele cases could be differentiated in terms of plasma levels of TNF-I and TNFR-II [19]. We had proposed that differential production of TNFRs could play a role in the development of lymphedema and emphasized the need to study TNF receptor polymorphism in the 2 diverse forms of chronic disease. In this study, we report analysis of genetic variability of TNFR-II and ET-1 genes in a large clinically well-defined cohort of hydrocele or elephantiasis cases. The TNFR-II gene consists of 10 exons and 9 introns and is located on chromosome 1p36.2 [20]. Our focus here is on a polymorphism at codon 196 (+676), exon 6 of the TNFR-II gene (rs1061622) that leads to an amino acid change of methionine to arginine. This polymorphism has been shown to affect the plasma-soluble TNFR-II levels [21]. To our knowledge, the present study is the first of its kind to address the genetic basis for development of hydrocele or elephantiasis, the 2 diverse forms of chronic disease in human filariasis.

MATERIALS AND METHODS

Study Participants and Clinical Characteristics
A total of 403 individuals were investigated, including 175 endemic control participants, 44 asymptomatic carriers, 89 hydrocele patients, and 95 elephantiasis patients residing in Mendashal, Kansapada, and Padanpur villages of Khurda district of Odisha State, India, a region that is highly endemic for bancroftian filariasis [6, 22]. Individuals were examined clinically for chronic disease manifestations of lymphatic filariasis as follows: patients presenting with persistent grade III lymphedema non-reversible on elevation with thickened skin for 5 years or more were recruited into the elephantiasis group [23]. Patients with scrotal swelling measuring >6 cm for 5 years or more were classified as hydrocele patients. Healthy individuals residing in the same villages without any history or current clinical symptoms and with no evidence of infection (confirmed by absence of circulating microfilariae or circulating filarial antigens) were considered endemic control participants in this study [19, 24]. Individuals generally free of overt clinical symptoms with circulating microfilariae of nocturnal periodicity were considered asymptomatic microfilariae carriers. Table 1 shows the characteristics of the study participants. About 3–4 mL of blood was obtained from all individuals in anticoagulant. Parasitological examination of individuals was performed by means of microscopic examination of Giemsa-stained finger prick blood smear samples (20 µL) obtained by night blood survey. On the basis of the parasitological and clinical manifestations, the patients were classified into the endemic control, asymptomatic microfilariae carrier, hydrocele, and elephantiasis groups. Only those with either hydrocele or elephantiasis were included in this study. Hydrocele patients and elephantiasis patients with demonstrable filarial infection (positive for microfilaremia or circulating filarial antigen) were excluded from the cohort. The prevalence of overt clinical manifestations was 9% (both elephantiasis and hydrocele) in this study area. About 39% of patients with hydrocele and 17% of patients with lymphedema were found to harbor filarial infection [6]. The institutional human ethical committee of the Regional Medical Research Centre, Bhubaneswar, India, approved the study.

Determination of Circulating Filarial Antigenemia
The level of circulating filarial antigens was measured with the Trop Bio ELISA test kit (Trop Bio Pvt Ltd) in accordance with the manufacturer’s protocol. In brief, 50 µL of the diluted plasma was added to plate wells in duplicate and the plate was incubated overnight. The optical density at 414 nm was recorded from the plasma samples. Antigen units were calculated with a standard curve from standards provided by the manufacturer.

316 • JID 2011:204 (15 July) • Panda et al
ET-1 (Ala288Ser) and TNFR-II (Met196Arg) Genotyping

Genomic DNA was extracted from the whole blood using the QIAamp DNA Blood Mini Kit (Qiagen), according to the manufacturer protocol. ET-1 (Ala288Ser) genotyping was performed by means of the polymerase chain reaction confronting 2 pairs primers method (PCR-CTPP) using predesigned primers [25]. PCR was carried out in a total volume of 20 μL using the following amplification protocol: denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 56°C for 40 seconds, and extension at 72°C for 50 seconds. Detection of the TNFR-II (Met196Arg) codon polymorphism was carried out by means of PCR followed by restriction enzyme digestion. Primer pairs used for detection of the TNFR-II (Met196Arg) polymorphism were 5′-ACT CTC CTA TCC TGC CTG CTG GT-3′ and 5′-TTC TGG AGT TGG CTG CGT GT-3′. PCR was performed using the following conditions: 95°C for 5 minutes followed by 35 cycles of 94°C for 1 minute, 64°C for 1 minute, and 72°C for 2 minutes. A final extension step was carried out at 72°C for 5 minutes. PCR products were digested with Nla III and visualized with 3.5% agarose gel electrophoresis. About 20% of the randomly selected samples were regenotyped for TNFR-II (Met196Arg) and ET-1 (Ala288ser) polymorphism, and the results were found to be 100% concordant, which ensured absence of genotyping error.

Table 1. Details of Study Population Analyzed for Endothelin-1 (ET-1), Tumor Necrosis Factor Receptor I (TNFR-I), and Tumor Necrosis Factor Receptor II (TNFR-II)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Endemic control participants (n = 175)</th>
<th>Hydrocele patients (n = 89)</th>
<th>Elephantiasis patients (n = 95)</th>
<th>Asymptomatic carriers (n = 44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male 86 89 37 28</td>
<td>Female 89 0 58 16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, mean years (range)</td>
<td>Male 38 (15–60) 34 (17–60) 45 (21–65) 28 (6–76)</td>
<td>Female 31 (20–56) ... 46 (19–70) 23 (8–45)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ET-1</td>
<td>Tested for genotype 100 70 84 40</td>
<td>Tested for phenotype 23 34 31 ...</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNFR-II</td>
<td>Tested for genotype 100 56 75 44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNFR-I</td>
<td>Tested for genotype 41 46 30 40</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ET-1 and TNFR-II Polymorphisms in Filariasis

Statistical Analysis

Statistical analyses were performed by using GraphPad Prism (version 5.01). The Fisher exact test was used to analyze the association of allele and genotype frequencies among different groups. Deviations from Hardy-Weinberg equilibrium were tested using the Web site http://www.ege.org/software/hwe-mr-calc.shtml. The unpaired Student t test was used for analyzing plasma ET-1 concentrations in different clinical categories and for the relationship between plasma ET-1 levels and ET-1 genotypes. Distributions of minor alleles of TNFR-II (Met196Arg) polymorphism in geographical populations were compared by means of the Fisher exact test. A P value of <.05 was considered to reveal a significant difference.

RESULTS

Plasma Levels of ET-1 in Chronic Human Filariasis

Of the 403 individuals investigated in the present study, plasma samples from only 88 were available for quantification of ET-1 levels. The ET-1 levels were significantly higher in hydrocele patients (2.588 μg/mL) than in elephantiasis patients (1.261 μg/mL) and endemic control participants (1.362 μg/mL) as shown in Figure 1. The ET-1 levels in endemic control participants and elephantiasis patients were comparable.

Phenotype–Genotype Relationship of Mutation

Figure 2 shows the association between plasma ET-1 levels and the ET-1 gene (Ala288Ser) mutation. ET-1 levels were significantly lower in participants with the mutation (heterozygous...
The ET-1 (Ala288Ser) Gene Polymorphism in Chronic Filariasis

The genotype and allele frequencies of ET-1 (Ala288Ser) were studied in a cohort of 294 participants belonging to the following groups: (1) endemic control participants, (2) microfilariaemic patients, (3) hydrocele patients, and (4) elephantiasis patients (Table 2). The ET-1 mutation (Ala288Ser) was significantly less frequent in hydrocele patients than in endemic control participants (odds ratio, 3.23) and elephantiasis patients (odds ratio, 2.15). This mutation frequencies in endemic control participants, microfilariaemic patients, and elephantiasis patients were comparable.

Genetic Polymorphism of TNFRs in Chronic Human Filariasis

We had earlier reported a correlation between plasma TNF and TNF receptors in acute and chronic filariasis [19]. The levels of the 2 TNF receptors types I and II were found to be inversely associated with the development of hydrocele and elephantiasis, respectively. The genetic basis of this differential production of TNF receptors was addressed in this study. The frequencies of the most commonly reported single-nucleotide polymorphism (SNP) for TNFR-I (+36 A/G) were comparable in patients with elephantiasis, patients with hydrocele, and endemic control participants (data not shown), suggesting that this polymorphism may play no significant role in development of chronic disease manifestations in human filariasis. Conversely, the genotype and allele distribution of TNFR-II (Met196Arg) polymorphism correlated significantly in patients with elephantiasis (Table 3). The wild genotype (Met196Met) was significantly more frequent in elephantiasis patients than in endemic control participants (odds ratio, 16.02) and hydrocele patients (odds ratio, 8.44). The distributions of the minor allele (196Arg) were comparable in endemic control participants and hydrocele patients (Table 3).

Association of TNFR-II and ET-1 Polymorphism in Males and Females

We analyzed the association of ET-1 and TNFR-II polymorphisms with chronic filariasis in males and females separately. In males, the ET-1 wild type (Ala288Ala) was significantly more frequent in hydrocele patients than in endemic control participants and asymptomatic carriers; in contrast, the prevalence of wild-type TNFR-II (Met196Met) was significantly higher in the elephantiasis group than in the endemic control group and asymptomatic carrier group. Since hydrocele is restricted to males, we compared the distribution of ET-1 and TNFR-II polymorphisms in the endemic control, asymptomatic carrier, and elephantiasis clinical categories in females. No significant difference was observed in distribution of ET-1 polymorphism among females of these 3 groups (data not shown).

Combined Distribution of TNFR-II (Met196Arg) and ET-1 (Ala288Ser) Polymorphism in Chronic Filariasis

Our study revealed an interesting polarization of ET-1 and TNFR-II genetic polymorphism in hydrocele and elephantiasis patients—the TNFR-II (Met196Arg) genotype was highly prevalent in hydrocele patients, and the ET-1 (Ala288Ser) genotype was highly prevalent in elephantiasis patients. Thus, a combined association analysis of both polymorphisms was undertaken and the results are shown in Table 4. A high frequency of elephantiasis was observed in patients (49%) with wild-type TNFR-II (Met196Met) and mutation in the ET-1 gene (Ala288Ser and Ser288Ser). In contrast, a significantly high frequency of

[Ala288Ser] or homozygous [Ser288Ser]) than in participants with the wild type (Ala288Ala).

**Figure 1.** Plasma levels of endothelin-1 (ET-1) in lymphatic filarial patients according to their clinical status (elephantiasis or hydrocele), compared with endemic control participants. Plasma concentrations (mean ± standard deviation) of ET-1 were measured, using a commercial kit (R & D Systems) from plasma of hydrocele patients (n = 34), elephantiasis patients (n = 31), and endemic control participants (n = 23). Mean plasma levels of ET-1 were significantly elevated in patients with hydrocele, compared with elephantiasis patients (P = .006) and endemic control participants (P = .02). EC, endemic control; ELE, elephantiasis; HYD, hydrocele.

**Figure 2.** Association between endothelin-1 (ET-1) (Ala288Ser) polymorphism and plasma ET-1 in filarial patients. Plasma concentrations (mean ± standard deviation) of ET-1 were measured, using a commercial kit, from plasma of chronic filarial patients (n = 45) and correlated with the genotype. Mean plasma levels of ET-1 were significantly elevated in samples from patients with wild type (Ala288Ala), compared with samples from patients with mutants (Ala288Ser and Ser288Ser) (P = .03).
Table 2. Genotype and Allele Distribution of Endothelin-1 (Ala288Ser) Polymorphism in Human Filariasis

<table>
<thead>
<tr>
<th>Genotype</th>
<th>EC</th>
<th>ELE</th>
<th>HYD</th>
<th>AS</th>
<th>P&lt;sup&gt;a&lt;/sup&gt; EC vs Hyd</th>
<th>OR&lt;sup&gt;b&lt;/sup&gt; (95% CI) EC vs HYD</th>
<th>P&lt;sup&gt;a&lt;/sup&gt; ELE vs HYD</th>
<th>OR&lt;sup&gt;b&lt;/sup&gt; (95% CI) ELE vs HYD</th>
<th>P&lt;sup&gt;a&lt;/sup&gt; AS vs HYD</th>
<th>OR&lt;sup&gt;b&lt;/sup&gt; (95% CI) AS vs HYD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala288Ala</td>
<td>16 (16)</td>
<td>15 (18)</td>
<td>28 (40)</td>
<td>7 (18)</td>
<td>&lt;.001</td>
<td>3.50 (1.71–7.17)</td>
<td>.004</td>
<td>3.07 (1.47–6.40)</td>
<td>.02</td>
<td>3.14 (1.22–8.09)</td>
</tr>
<tr>
<td>Ala288Ser</td>
<td>72 (72)</td>
<td>53 (63)</td>
<td>31 (44)</td>
<td>28 (70)</td>
<td>&lt;.001</td>
<td>3.23 (1.7–6.15)</td>
<td>.02</td>
<td>2.15 (1.13–4.10)</td>
<td>.01</td>
<td>0.34 (0.15–0.78)</td>
</tr>
<tr>
<td>Ser288Ser</td>
<td>12 (12)</td>
<td>16 (19)</td>
<td>11 (16)</td>
<td>5 (12)</td>
<td>.50</td>
<td>0.73 (0.3–1.76)</td>
<td>.67</td>
<td>1.26 (0.54–2.93)</td>
<td>.78</td>
<td>0.76 (0.24–2.38)</td>
</tr>
<tr>
<td>Allele</td>
<td>2n = 200</td>
<td>2n = 168</td>
<td>2n = 140</td>
<td>2n = 80</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ala288</td>
<td>104 (52)</td>
<td>83 (49)</td>
<td>87 (62)</td>
<td>42 (53)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>288Ser</td>
<td>96 (48)</td>
<td>85 (51)</td>
<td>53 (38)</td>
<td>38 (47)</td>
<td>.08</td>
<td>1.51 (0.97–2.35)</td>
<td>.03</td>
<td>1.68 (1.06–2.65)</td>
<td>.20</td>
<td>1.48 (0.85–2.59)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of participants unless otherwise specified. AS, asymptomatic carrier; CI, confidence interval; EC, endemic control; ELE, elephantiasis patient; HYD, hydrocele patient; OR, odds ratio.

<sup>a</sup> Determined by Fisher exact test.

Table 3. Genotype and Allele Distribution of Tumor Necrosis Factor Receptor II (TNFR-II) (Met196Arg) Polymorphism in Human Filariasis

<table>
<thead>
<tr>
<th>Genotype</th>
<th>EC</th>
<th>ELE</th>
<th>HYD</th>
<th>AS</th>
<th>P&lt;sup&gt;a&lt;/sup&gt; EC vs ELE</th>
<th>OR&lt;sup&gt;b&lt;/sup&gt; (95% CI) EC vs ELE</th>
<th>P&lt;sup&gt;a&lt;/sup&gt; ELE vs HYD</th>
<th>OR&lt;sup&gt;b&lt;/sup&gt; (95% CI) ELE vs HYD</th>
<th>P&lt;sup&gt;a&lt;/sup&gt; AS vs ELE</th>
<th>OR&lt;sup&gt;b&lt;/sup&gt; (95% CI) AS vs ELE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met196Met</td>
<td>7 (7)</td>
<td>41 (55)</td>
<td>7 (12.5)</td>
<td>4 (9)</td>
<td>&lt;.001</td>
<td>16.02 (6.56–39.12)</td>
<td>&lt;.001</td>
<td>8.44 (3.38–21.04)</td>
<td>&lt;.001</td>
<td>12.06 (3.91–37.11)</td>
</tr>
<tr>
<td>Met196Arg</td>
<td>76 (76)</td>
<td>29 (39)</td>
<td>41 (73)</td>
<td>33 (75)</td>
<td>&lt;.001</td>
<td>5.02 (2.61–9.65)</td>
<td>&lt;.001</td>
<td>4.34 (2.04–9.20)</td>
<td>&lt;.001</td>
<td>4.75 (2.08–10.87)</td>
</tr>
<tr>
<td>Arg196Arg</td>
<td>17 (17)</td>
<td>5 (6)</td>
<td>8 (14.5)</td>
<td>7 (16)</td>
<td>.06</td>
<td>2.86 (1.01–8.17)</td>
<td>0.24</td>
<td>2.33 (0.72–7.57)</td>
<td>.12</td>
<td>0.38 (0.11–1.27)</td>
</tr>
<tr>
<td>Allele</td>
<td>2n = 200</td>
<td>2n = 150</td>
<td>2n = 112</td>
<td>2n = 88</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Met196</td>
<td>90 (45)</td>
<td>111 (74)</td>
<td>55 (49%)</td>
<td>41 (47)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>196Arg</td>
<td>110 (55)</td>
<td>39 (26)</td>
<td>57 (51)</td>
<td>47 (53)</td>
<td>&lt;.001</td>
<td>3.48 (2.19–5.50)</td>
<td>&lt;.001</td>
<td>2.95 (1.75–4.97)</td>
<td>&lt;.001</td>
<td>3.26 (1.87–5.69)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of participants unless otherwise specified. AS, asymptomatic carrier; CI, confidence interval; EC, endemic control; ELE, elephantiasis patient; HYD, hydrocele patient; OR, odds ratio.

<sup>a</sup> Determined by Fisher exact test.
hydrocele (38%) was observed in patients with mutation in TNFR-II and wild-type ET-1 (Table 4). Absence of mutation in both TNFR-II and ET-1 genes was uncommon in our cohort of patients developing chronic filarial disease, and the presence of mutation in both genes failed to differentiate the 2 diverse clinical forms (Table 4). The results shown in Table 4 thus offer a predictive value for these 2 mutations in the context of susceptibility to develop one or the other form of chronic disease.

**DISCUSSION**

Infection with *W. bancrofti* leads to the development of lymphedema and/or hydrocele in only a small proportion of the population in filarial endemic areas [3–6]. The variable outcome of chronic manifestations has been attributed to differences in immunological responsiveness to filarial parasites in exposed populations. Although in general the immunological responses are genetically regulated [26], the contribution of host genetic factors toward development of the 2 diverse forms of chronic disease manifestations in filariasis (elephantiasis and hydrocele) is yet to be reported. A recent report has suggested an association between VEGF-A gene promoter polymorphism and the development of hydrocele in patients infected with *W. bancrofti* [11]. Here we report polymorphism of the ET-1 and TNFR-II genes and their association with development of one or the other form of chronic disease manifestations in human bancroftian filariasis. To our knowledge, the effect of mutation of the ET-1 gene at coding region 288 (Ala288Ser) on plasma levels has not been reported in the literature thus far. Six SNPs of ET-1 are known, and the most commonly studied SNP (Lys198Asn) has been shown not to correlate with plasma ET-1 levels consistently [27–29]. The ET-1 SNP (Ala288Ser) investigated here has not been widely studied in other diseases. One study in a Japanese population analyzed this mutation and found no significant association with hypertension [25]. Our results indicated significantly low levels of plasma ET-1 in participants with mutation (Ala288Ser and Ser288Ser), in comparison with those with wild type (Ala288Ala). Since the observed mutation is in the coding region, we propose that Ala to Ser substitution could have contributed to enhance the degradation of plasma ET-1, leading to lower levels of plasma ET-1.

Hydrocele patients displayed significantly higher plasma ET-1 levels when compared with other groups, which suggests a role for ET-1 in the development of hydrocele. ET-1 is known to promote extravasations of fluid and plasma proteins from the blood vessels into the surrounding tissues, and hydrocele is characterized by the accumulation of fluid in the tunica vaginalis, the sac covering the testis, and gradual increases of swelling over a period of time. The observed higher frequency of ET-1 (Ala288Ala) genotype could thus be a contributing factor in the pathogenesis of hydrocele by facilitating the accumulation of plasma proteins and fluid in the tunica vaginalis.

Molecules related to immune response have been attributed as a cause of development of chronic disease in human filariasis. We previously reported a role for TNF [17] and TNF receptors [19] in the development of disease manifestations in human filariasis. Plasma levels of type I and type II TNF receptors were found to be correlated in hydrocele/elephantiasis patients, and in this study we have addressed the genetic basis of this differential production of TNF receptors. We could not determine the plasma levels of TNFR-II, because insufficient samples were available. This mutation of TNFR-II has been reported to decrease plasma TNFR-II levels [21]. The major allele (196Met) associated with higher plasma levels of TNFR-II [21] was more prevalent in elephantiasis patients than in hydrocele patients. Significantly increased plasma levels of TNFR-II have been implicated in peritoneal fibrosis [30], granuloma formation in murine schistosomiasis [31], and in elephantiasis [19]. It has been suggested that in filariasis, granulomatous nodule formation impairs lymphatic flow, predisposing the host to secondary bacterial infections, which results in fibrosis, lymphatic obstruction leading to lymphedema [7]. We propose that the higher frequency of the Met196Met genotype of TNFR-II observed in elephantiasis patients in this study could be a predisposing factor for the development of elephantiasis. More importantly, when the ET-1 and TNFR-II variant genotypes were combined for analysis (Table 4), a 23-fold increase in the risk of elephantiasis development was associated with TNFR-II (Met196Met) genotype and mutation in ET-1 (Ala288Ser + Ser288Ser). Conversely, participants with ET-1 wild type (Ala288Ala) and mutation for TNFRII (Met196Arg and Arg196Arg) were predisposed to develop hydrocele (odds ratio, 6.64), suggesting a clear genetic basis for the development of one or the other form of chronic disease in human bancroftian filariasis.

The current study also provided insights into the role played by filarial nematodes in the selection of immune response–related genes in the population. ET-1 (Ala288Ser) is not a widely

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**Table 4. Combined Genotype Frequencies of Tumor Necrosis Factor Receptor II (TNFR-II) (Met196Arg) and Endothelin-1 (ET-1) (Ala288Ser) Polymorphisms in Human Filariasis**

<table>
<thead>
<tr>
<th>Genotype Combination</th>
<th>ELE (n = 71)</th>
<th>HYD (n = 50)</th>
<th>P*</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild/Wild</td>
<td>5 (8)</td>
<td>3 (6)</td>
<td>&gt;.99</td>
<td>1.18 (0.27–5.21)</td>
</tr>
<tr>
<td>Wild/Mutant</td>
<td>35 (49)</td>
<td>2 (4)</td>
<td>&lt;.001</td>
<td>23.33 (5.26–103.50)</td>
</tr>
<tr>
<td>Mutant/Wild</td>
<td>6 (8)</td>
<td>19 (38)</td>
<td>&lt;.001</td>
<td>6.64 (2.41–18.28)</td>
</tr>
<tr>
<td>Mutant/Mutant</td>
<td>25 (35)</td>
<td>26 (52)</td>
<td>.09</td>
<td>0.50 (0.24–1.05)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of participants unless otherwise specified. CI, confidence interval; ELE, Elephantiasis; HYD, Hydrocele; OR, odds ratio.

* Fisher exact test was used to determine the combined effect of TNFR-II (Met196Arg) and ET-1 (Ala288Ser) polymorphisms.

b TNFR-II (Met196Met).
c ET-1 (Ala288Ala).
d ET-1 (Ala288Ser + Ser288Ser).
e TNFR-II (Met196Arg + Arg196Arg).
investigated SNP, and one report in a Japanese population revealed a prevalence of 30.7% mutant alleles [25], whereas in our population (endemic control participants) the frequency was found to be 48%. The prevalence of the TNFR-II minor allele (196Arg) was found to be substantially higher in endemic control participants (55%), compared with that in other reports, including one from north India [32, 33]. The distribution of ET-1 (Ala288Ser) and TNFR-II (Met196Arg) polymorphisms in our cohort did not display Hardy-Weinberg equilibrium, deviation from which in a population has been attributed to selection pressure and/or population stratifications, and so forth [34]. The participants in the present study were members of an ethnic population living in villages, which suggests that the observed deviation from Hardy-Weinberg equilibrium was probably due to selection pressure, confirming the proposal that infection with helminths could select for specific cytokine genes [35].

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