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BRIEF COMMUNICATION

Polymorphisms of innate immunity genes and susceptibility to lymphatic filariasis

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We examined 906 residents of an area of Papua New Guinea where bancroftian filariasis is endemic for genetic polymorphisms in three innate immunity genes suspected of contributing to susceptibility to infection and lymphatic pathology. Active infection was confirmed by the presence of blood-borne microfilariae and circulating filarial antigen in plasma. Disease was ascertained by physical examination for the presence of overt lymphedema (severe swelling of an arm or leg) or hydrocele. There was no association of infection status, lymphedema of an extremity, or hydrocele with chitotriosidase genotype (CHIT1). Polymorphisms of toll-like receptor-2 and toll-like receptor-4 genes (TLR4 A896G; TLR2 T2178A, G2258A) were not detected (N=200–625 individuals genotyped) except for two individuals heterozygous for a TLR2 mutation (C2029 T). These results indicate that a CHIT1 genotype associated previously with susceptibility to filariasis in residents of southern India and TLR2 and TLR4 polymorphisms do not correlate with infection status or disease phenotype in this Melanesian population.
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Lymphatic filariasis is a mosquito-transmitted helminth infection endemic in sub-Saharan Africa, Asia, tropical islands of the Pacific Ocean, and focal areas of Latin America. Humans are infected when mosquitoes inoculate infective larvae into the skin that develop into lymphatic-dwelling adult worms and produce millions of microfilariae that circulate in the bloodstream. The life cycle is completed when microfilariae are taken up in the blood meal of the mosquito vector, where they develop into infective larvae over 10–14 days. The pathological consequences of *Wuchereria bancrofti* infection (there are also two less common filarial species that infect humans, *Brugia malayi* and *B. timori*) are mainly due to lymphatic dysfunction clinically manifested as lymphedema of the extremities or elephantiasis and disfigurement of the genitalia, especially hydroceles in men.

Infection and disease due to *W. bancrofti* have a characteristic distribution in populations living in endemic areas. The frequency of infection is generally higher in adults than children, consistent with the notion that the parasite burden increases with cumulative exposure to mosquito-borne larvae. The prevalence of overt clinical manifestations among adult residents of endemic areas is usually less than 10% despite the fact that most individuals are presumably inoculated with infective larvae throughout life. In high transmission areas of Papua New Guinea, for example, community-specific infection rates documented by the presence of blood-borne microfilariae and filarial antigenemia range from 50% to over 80% in persons older than 20 years of age, whereas fewer than 10% have lymphedema of the extremities.[1] This heterogeneity in infection and disease has been attributed to differences in antigen-specific T-cell immunity,[2] spatial variables such as exposure to mosquitoes containing infective larvae,[3] and genetic factors.[4,5] Progress on understanding the contribution of host genetics to susceptibility to human lymphatic filariasis has come slowly, and primarily been related to the suggestion that HLA antigens are involved, although this may not hold true in all populations.[6–8] More recently, a study of residents of southern India suggested an association between a duplication mutation in exon 10 of the chitotriosidase gene (*CHIT1*) and susceptibility to lymphatic disease and microfilaremia.[4] There was no association between the *CHIT1* mutant allele and tropical eosinophilia, a less common asthma-like condition associated with lymphatic filarial infection. Chitotriosidase is expressed by macrophages and putatively functions to degrade chitin-containing pathogens such as filarial parasites.[9] Gaucher's disease results from a deficiency in glucocerebrosidase activity and is characterized by overproduction of chitotriosidase.[10,11] In contrast, the 24 bp duplication in exon 10 of *CHIT1* leads to abnormal mRNA splicing and production of an enzymatically inactive truncated protein. The allelic frequency in a healthy Dutch population was reported to be 0.239, with similar frequencies in Ashkenazi Jewish and Indonesian populations.[9] Similarly, an allelic frequency of 0.236 was found in a cohort of Israeli patients with Candida sepsis[12] and 0.258 in a Spanish population with hyperlipidemia.[13] In the current study, we determined whether there was an association between the

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CHIT1 duplication allele and infection and disease status in residents of a *W. bancrofti* endemic area of Papua New Guinea. As endotoxin-like molecules produced by endosymbiotic *Wolbachia* contained within filarial nematodes have recently been implicated in the pathogenesis of disease in filariasis,[14,15] we also examined the association of infection and disease with selected polymorphisms of toll-like receptor 2 and 4 (*TLR2* and *TLR4*). Proteins encoded by these innate immune receptor genes are thought to be involved in generation of proinflammatory responses to endotoxin and related microbial ligands.[16, 17]

A total of 906 individuals were included in the study. The methodology used for genotyping is summarized in Table 1. The frequency of the mutant allele (24 bp duplication) *CHIT1* genotype was 0.121 (219/1812). A total of 205 people were heterozygous (22.6%) and seven were homozygous (0.8%). There was no significant association between the *CHIT1* allele genotype and infection status confirmed by blood-borne microfilariae or filarial antigenemia (Table 2). The *CHIT1* genotype was not associated with lymphatic pathology defined by the presence of severe lymphedema of the arm or leg (grade 2 or higher according to the World Health Organization criteria) or advanced hydrocele detected by physical examination (Table 3).[18] All of the individuals genotyped for polymorphisms in *TLR4* and *TLR2* were homozygous for the wild-type allele (Table 4) except for two individuals heterozygous for a *TLR2* mutation (C2029 T). Therefore, no statistical correlation could be made between these mutations and filarial infection or disease.

The results of the current study differ from the report of Choi *et al*[4] who found a significant association between susceptibility to filariasis and homozygosity ($P = 0.013$) for the *CHIT1* mutant allele in southern India. The difference in the results of the two studies is more striking in view of the fact that 180 individuals from southern India were examined *vs* 906 from Papua New Guinea. These differences may be related to several factors. First, the overall frequency of the 24 bp duplication allele in Papua New Guinea was lower than in southern India (0.121 *vs* 0.442). The frequency in Papua New Guinea is similar to that of healthy African-Americans and North American Caucasians.[4] Second, the definition of infection and disease phenotypes in the two studies differed in several respects. In Papua New Guinea, none of the subjects had previously been treated with antifilarial drugs (all were residents of a remote area who subsequently participated in trials to assess the impact of mass treatment with antifilarial medications),[19] and infection status was determined by measurements of both blood-borne microfilariae and filarial antigen in plasma. The study from India found a statistically significant association between the chitotriosidase mutation and a combined measure of presence of microfilaremia and antigenemia and chronic lymphatic disease. No association was found when these variables were analyzed separately, as in our study. Additionally, the south Indian study found a decrease in disease susceptibility with heterozygous individuals compared to homozygous wild type and *CHIT*1 combined ($P=0.034$). This may be an artifact of the relatively small number of uninfected individuals considered in that analysis ($N=67$). Third, Papua New Guinea study subjects are members of small linguistic groups in East Sepik Province (Drat and Urim), whereas the south Indian study likely included subjects of multiple ethnicities. Finally, although the relatively low frequency of homozygosity for the allele in the Papua New Guinea population would limit the power to detect disease associations, our study did have adequate power to detect the effect of heterozygosity based on the level of difference reported by Choi *et al*.[4]

Polymorphisms in *TLR4* (A896G) and *TLR2* (C2029 T, T2178A, G2258A) have previously been described (Genbank U88878).[20–22] None of the *TLR4* and only two of the *TLR2* polymorphisms were detected in the Papua New Guinea study population (Table 4). A polymorphism in exon 4 of *TLR4* resulting in a Thr399lle amino-acid substitution has been reported and linked to an Asp299Gly (A896G) amino-acid substitution.[22,23] Genotyping for the former polymorphism was not performed in this study, as the A896G substitution was not observed. Allele frequencies of the *TLR4* polymorphism we examined here have been reported to be 0.033–0.080 in Caucasian populations of Europe and North America;[22,24] only the wild-type TLR4 allele was observed in

Table 1 Genotyping conditions

Gene		*Primers*	*Annealing temp. (°C)*	*Enzyme digest*
CHIT1	up	5′-AGCTATCTGAAGCAGAAG-3′	55	None
(Ref. Genbank NM_003465)	dn	5′-GGAGAAGCCGGCAAAGTC-3′		
TLR4 (A896G)	up	5′-GATTAGCATACTTAGACTACTACCTCCATG-3′	55	*NcoI*
(Ref Genbank AF177765.1)	dn	5′-GATCAACTTCTGAAAAAGCATTCCCAC-3′		
TLR2(T2178A)	up	5′-TGGTGCAAGTATGAACTGGAC-3′	60	*EarI*
(Ref Genbank NM_003264.2)	dn	5′-TTGCAGAAGCGCTGGGGAATGGC-3′		
TLR2 (C2029 T)	up	5′-TATGGTCCAGGAGCTGGAGA-3′	55	*AciI*
(Ref. Genbank NM_003264.2)	dn	5′-TGACATAAAGATCCCAACTAGACAA-3′		
TLR2 (G2258A)	up	5′-TATGGTCCAGGAGCTGGAGA-3′	55	*PstI*
(Ref Genbank NM_003264.2)	dn	5′-TGACATAAAGATCCCAACTAGACAA-3′		

Whole blood was obtained from a population in the Dreikikir District of the East Sepik Province, PNG in 1993, with approval of the Institutional Review Board for Human Investigation at Case Western Reserve University and the Medical Advisory Committee of the government of Papua New Guinea. Genomic DNA was extracted using commercial kits (Qiagen, Hilden, Germany). After amplification at the annealing temperature indicated in the table, PCR products were digested with the appropriate restriction enzyme (New England Biolabs, Beverly, MA, USA) and the PCR product (CHIT1) or enzyme digest separated by gel electrophoresis.

Table 2 *CHIT1* mutation frequency and infection status

	Genotype distribution			
	wt/wt	*wt/mut*	*mut/mut*	*P-value*
Microfilaremia[a]				
Total	644 (75.9%)	198 (23.3%)	6 (0.7%)	
Present	302 (77.6%)	86 (22.1%)	1 (0.3%)	0.25
Absent	342 (74.5%)	112 (24.4%)	5 (1.1%)	
Antigenemia[b]				
Total	345 (73.9%)	120 (25.7%)	2 (0.4%)	
Present	70 (72.9%)	25 (26.0%)	1 (1.0%)	0.58
Absent	275 (74.1%)	95 (25.6%)	1 (0.3%)	
Antigen level[c]				
High	206 (73.3%)	74 (26.3%)	1 (0.4%)	
Low	69 (76.7%)	21 (23.3%)	0	0.80
Negative	70 (72.9%)	25 (26.0%)	1 (1.0%)	

[a]Microfilaremia determined by light microscopy on 1.0 ml of venous blood drawn between 10 pm and 2 am subjected to Nuclepore filtration.[30]
[b]Antigenemia defined as circulating antigen levels above 32 antigen units (AU) as quantitatively determined by the Og4C3 ELISA previously described.[31]
[c]Antigen levels are defined as high (>512 AU), low (>32 AU; ≤512 AU), and negative (≤32 AU). Associations among genotype and infection status were calculated by means of Pearson χ^2 tests using SAS statistical software (Cary, NC, USA). $P<0.05$ was considered to be significant. All ages included in this analysis.

Table 3 *CHIT1* allele frequency and lymphatic filariasis disease status

	Genotype Distribution			
	wt/wt	*wt/mut*	*mut/mut*	*P-value*
Clinical disease[a]				
Total	694/906 (76.60%)	205/906 (22.63%)	7/906 (0.77%)	
Present	51 (75.0%)	17 (25.0%)	0	0.66
Absent	643 (76.8%)	188 (22.4%)	7 (0.8%)	
LE edema				
Present	24 (70.6%)	10 (29.4%)	0	0.56
Absent	670 (76.8%)	195 (22.4%)	7 (0.8%)	
UE edema				
Present	4 (100.0%)	0	0	0.54
Absent	690 (76.5%)	205 (22.7%)	7 (0.8%)	
Hydrocele[b]				
Present	23 (79.3%)	6 (20.7%)	0	0.52
Absent	96 (69.1%)	42 (30.2%)	1 (0.7%)	

[a]Clinical disease was defined to be the presence of any of the following: leg edema, arm edema (age >21 years) or hydrocele.
[b]Hydrocele evaluated in men age ≥16 years. Statistical analysis was performed as described in Table 2.

Table 4 Frequencies of Toll-like receptor gene polymorphisms

TLR gene polymorphism	*Genotype*	*Freq. (%)*
TLR4 (A896G)	*wt/wt*	625/625 (100%)
	wt/mut	0/625
	mut/mut	0/625
TLR2 (T2178A)	*wt/wt*	200/200 (100%)
	wt/mut	0/200
	mut/mut	0/200
TLR2 (C2029T)	*wt/wt*	264/266 (99.2%)
	wt/mut	2/266 (0.8%)
	mut/mut	0/266
TLR2 (G2258A)	*wt/wt*	283/283 (100%)
	wt/mut	0/283
	mut/mut	0/283

an Asian population.[25] There is only limited data on the frequency of the *TLR2* mutations in various populations; the C2029 T allele was found at a frequency of 0.222 in a subset of 45 patients with lepromatous leprosy and was not detected in tuberculous leprosy patients or uninfected Korean controls,[17] the G2258A substitution was detected in 3% of 110 healthy North American controls.[20] Given the presumed origin of extant Melanesians from ancestral Asian populations,[26] it might be expected that the frequency of the *TLR4* genotypes studied here would be extraordinarily low. It should be noted however that these data do not exclude the possibility that other *TLR2* or *TLR4* polymorphisms are present.

There is considerable variability in the frequency of genetic polymorphisms in people of different ethnicities.[27]

Cultural and ethnic divisions in Papua New Guinea may, therefore, result in genetic heterogeneity and introduce possibilities that responses to infectious diseases might vary from one population to the next within this small country. On the other hand, founder effects originating from the time of initial human settlements in Papua New Guinea may be responsible for genetic homogeneity.[28,29] Additional genotyping studies of *CHIT1*, *TLR2*, *TLR4* and other candidate genes involved in susceptibility to lymphatic filariasis will be required to determine their contribution to the heterogeneous pattern of infection and disease in filariasis in Papua New Guinea and other endemic areas.

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References

1 Tisch DJ, Hazlett FE, Kastens W, Alpers MP, Bockarie MJ, Kazura JW. Ecologic and biologic determinants of filarial antigenemia in bancroftian filariasis in Papua New Guinea. *J Infect Dis* 2001; **184**: 898–904.
2 King CL, Ottesen EA, Nutman TB. Cytokine regulation of antigen-driven immunoglobulin production in filarial parasite infections in humans. *J Clin Invest* 1990; **85**: 1810–1815.
3 Kazura JW, Bockarie M, Alexander N *et al*. Transmission intensity and its relationship to infection and disease due to *Wuchereria bancrofti* in Papua New Guinea. *J Infect Dis* 1997; **176**: 242–246.
4 Choi EH, Zimmerman PA, Foster CB *et al*. Genetic polymorphisms in molecules of innate immunity and susceptibility to infection with *Wuchereria bancrofti* in South India. *Genes Immun* 2001; **2**: 248–253.
5 Garcia A, Abel L, Cot M *et al*. Genetic epidemiology of host predisposition microfilaraemia in human loiasis. *Trop Med Int Health* 1999; **4**: 565–574.
6 Chan SH, Dissanayake S, Mak JW *et al*. HLA and filariasis in Sri Lankans and Indians. *Southeast Asian J Trop Med Public Health* 1984; **15**: 281–286.
7 Yazdanbakhsh M, Sartono E, Kruize YC *et al*. HLA and elephantiasis in lymphatic filariasis. *Hum Immunol* 1995; **44**: 58–61.
8 Yazdanbakhsh M, Abadi K, de Roo M *et al*. HLA and elephantiasis revisited. *Eur J Immunogenet* 1997; **24**: 439–442.
9 Boot RG, Renkema GH, Verhoek M *et al*. The human chitotriosidase gene. Nature of inherited enzyme deficiency. *J Biol Chem* 1998; **273**: 25680–25685.
10 Hollak CE, van Weely S, van Oers MH, Aerts JM. Marked elevation of plasma chitotriosidase activity. A novel hallmark of Gaucher disease. *J Clin Invest* 1994; **93**: 1288–1292.
11 Giraldo P, Cenarro A, Alfonso P *et al* . Chitotriosidase genotype and plasma activity in patients type 1 Gaucher's disease and their relatives (carriers and non carriers). *Haematologica* 2001; **86**: 977–984.
12 Masoud M, Rudensky B, Elstein D, Zimran A. Chitotriosidase deficiency in survivors of Candida sepsis. *Blood Cells Mol Dis* 2002; **29**: 116–118.
13 Canudas J, Cenarro A, Civeira F *et al*. Chitotriosidase genotype and serum activity in subjects with combined hyperlipidemia: effect of the lipid-lowering agents, atorvastatin and bezafibrate. *Metabolism* 2001; **50**: 447–450.
14 Taylor MJ, Cross HF, Bilo K. Inflammatory responses induced by the filarial nematode *Brugia malayi* are mediated by lipopolysaccharide-like activity from endosymbiotic *Wolbachia* bacteria. *J Exp Med* 2000; **191**: 1429–1436.
15 Saint Andre A, Blackwell NM, Hall LR *et al*. The role of endosymbiotic *Wolbachia* bacteria in the pathogenesis of river blindness. *Science* 2002; **295**: 1892–1895.
16 Kaisho T, Akira S. Critical roles of Toll-like receptors in host defense. *Crit Rev Immunol* 2000; **20**: 393–405.
17 Kang TJ, Lee SB, Chae GT. A polymorphism in the toll-like receptor 2 is associated with IL-12 production from monocyte in lepromatous leprosy. *Cytokine* 2002; **20**: 56–62.
18 WHO. *Lymphatic filariasis: the disease and its control* Fifth Report of the WHO Expert Committee on Filariasis. Technical Report Series, No. 821, 1992.
19 Bockarie MJ, Tisch DJ, Kastens W *et al*. Mass treatment to eliminate filariasis in Papua New Guinea. *N Engl J Med* 2002; **347**: 1841–1848.
20 Lorenz E, Mira JP, Cornish KL, Arbour NC, Schwartz DA. A novel polymorphism in the toll-like receptor 2 gene and its potential association with staphylococcal infection. *Infect Immun* 2000; **68**: 6398–6401.
21 Kang TJ, Chae GT. Detection of Toll-like receptor 2 (TLR2) mutation in the lepromatous leprosy patients. *FEMS Immunol Med Microbiol* 2001; **31**: 53–58.
22 Arbour NC, Lorenz E, Schutte BC *et al*. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet* 2000; **25**: 187–191.
23 Lorenz E, Mira JP, Frees KL, Schwartz DA. Relevance of mutations in the TLR4 receptor in patients with gram-negative septic shock. *Arch Intern Med* 2002; **162**: 1028–1032.
24 Read RC, Pullin J, Gregory S *et al*. A functional polymorphism of toll-like receptor 4 is not associated with likelihood or severity of meningococcal disease. *J Infect Dis* 2001; **184**: 640–642.
25 Okayama N, Fujimura K, Suehiro Y *et al*. Simple genotype analysis of the Asp299Gly polymorphism of the Toll-like receptor-4 gene that is associated with lipopolysaccharide hyporesponsiveness. *J Clin Lab Anal* 2002; **16**: 56–58.
26 Ballinger SW, Schurr TG, Torroni A *et al*. Southeast Asian mitochondrial DNA analysis reveals genetic continuity of ancient mongoloid migrations. *Genetics* 1992; **130**: 139–152.
27 Cavalli-Sforza LL, Menozzi P, Piazza A. *The History and Geography of Human Genes*. Princeton University Press: Princeton, NJ, 1994.
28 Mack SJ, Bugawan TL, Moonsamy PV *et al*. Evolution of Pacific/Asian populations inferred from HLA class II allele frequency distributions. *Tissue Antigens* 2000; **55**: 383–400.
29 Bugawan TL, Mack SJ, Stoneking M, Saha M, Beck HP, Erlich HA. HLA class I allele distributions in six Pacific/Asian populations: evidence of selection at the HLA-A locus. *Tissue Antigens* 1999; **53**: (4 Part 1): 311–319.
30 Desowitz RS, Southgate BA. Studies of filariasis in the Pacific. 2. The persistence of microfilaraemia in diethylcarbamazine treated populations of Fiji and Western Samoa: diagnostic application of the membrane-filtration technique. *Southeast Asian J Trop Med Public Health* 1973; **4**: 179–183.
31 More SJ, Copeman DB. A highly specific and sensitive monoclonal antibody-based ELISA for the detection of circulating antigen in bancroftian filariasis. *Trop Med Parasitol* 1990; **41**: 403–406.