Available online at www.sciencedirect.com
ScienceDirect
Infection, Genetics and Evolution 7 (2007) 743–747
Infection, Genetics and Evolution
www.elsevier.com/locate/meegid

Short communication

Chitotriosidase deficiency is not associated with human hookworm infection in a Papua New Guinean population

Andrew J. Hall [a,1], Rupert J. Quinnell [b,1], Andrew Raiko [c], Moses Lagog [c], Peter Siba [c], Shaun Morroll [a], Franco H. Falcone [a,*]

[a] *Immune Modulation Research Group, School of Pharmacy, University of Nottingham, Nottingham NG7 2RD, United Kingdom*
[b] *Institute of Integrative and Comparative Biology, University of Leeds, Leeds LS2 9JT, United Kingdom*
[c] *Papua New Guinea Institute of Medical Research, PO Box 60, Goroka EHP 441, Papua New Guinea*

Received 23 March 2007; received in revised form 27 June 2007; accepted 23 July 2007
Available online 28 July 2007

Abstract

Human chitotriosidase (*CHIT1*) is a chitinolytic enzyme with suggested anti-fungal properties. Previous studies have suggested that chitotriosidase may also protect individuals against filarial nematode infections and malaria. A mutant allele, which renders chitotriosidase unstable and enzymatically inactive, is found at a frequency of >20% in Caucasians and other populations. This allele is found at much lower frequency in parts of West Africa where malarial and intestinal helminth infections are endemic. Here, we investigate whether there is a significant association between chitotriosidase genotype and the intensity of hookworm infection in 693 individuals from five villages in Papua New Guinea. Individuals were genotyped for chitotriosidase using a PCR-based assay. There was no association between *CHIT1* genotype and the intensity of hookworm infection as determined by faecal egg counts. The frequency of the mutant allele was 0.251, very similar to that found in non-endemic countries. The extent of geographical variation in allele frequencies across worldwide populations was not high ($F_{st} = 0.11$), and does not provide evidence for directional selection at this locus between different geographical areas. We conclude that the *CHIT1* genotype does not play a crucial role in protection against hookworm infection. This does not correlate with a previous study that linked the mutant *CHIT1* genotype to filariasis susceptibility. The possible reasons for this discrepancy are discussed.

Keywords: Chitotriosidase; *CHIT1* mutation; *Necator americanus*; Malaria; Papua New Guinea

1. Introduction

Chitotriosidase is a chitinolytic enzyme which was recently identified in man and is synthesised in large quantities by activated macrophages (Hollak et al., 1994). As humans lack endogenous chitin, a role for chitotriosidase is not fully understood. However the anti-fungal action of homologous plant chitinases has led to the suggestion that chitotriosidase may degrade chitin containing pathogens (Boot et al., 1998). The chitotriosidase gene (*CHIT1*) consists of 12 exons located on chromosome 1q31–32 (Boot et al., 1998). A 24 base pair duplication in exon 10 leads to the activation of a cryptic 3′ splice site that results in an abnormally spliced mRNA with an inframe 87 nucleotide deletion (Boot et al., 1998). The mutant protein lacks amino acids 344–372 that are required for the formation of the TIM-barrel catalytic core (Boot et al., 1998; Fusetti et al., 2002). Macrophages from chitotriosidase deficient individuals express only small amounts of mRNA and secrete virtually no chitotriosidase protein (Boot et al., 1998).

In Dutch and Ashkenazi Jewish populations 6% of individuals are homozygous for the mutant allele, whereas 35% and 34%, respectively, are heterozygous carriers (Boot et al., 1998) with a mutant allele frequency of 0.23. A similar allelic frequency is present in Portugal (0.22), Sicily (0.27) and Sardinia (0.21) (Malaguarnera et al., 2003). However in two West African countries, Benin and Burkina Faso, that are mesoendemic for *Plasmodium falciparum* malaria and endemic for gastrointestinal helminths, a total absence of the homozygous mutation and significant reduction in heterozygous individuals has been reported, with mutant allele frequency

* Corresponding author. Tel.: +44 115 84 66073; fax: +44 115 95 15102.
E-mail address: Franco.Falcone@nottingham.ac.uk (F.H. Falcone).
[1] These authors contributed equally to this work.

1567-1348/$ – see front matter © 2007 Published by Elsevier B.V.
doi:10.1016/j.meegid.2007.07.010

0.00 and 0.02, respectively (Malaguarnera et al., 2003). This observation led to the hypothesis that chitotriosidase may be involved in resistance to protozoan or helminth infections common in tropical countries (Malaguarnera et al., 2003).

Parasitic nematodes are known to contain chitin, and are thus a potential target of human chitotriosidase. Chitin is found in the egg-shell of both free-living and parasitic nematodes, and in the microfilarial sheath surrounding the first-stage larvae of filarial nematodes (Fuhrman and Piessens, 1985). More recently, chitin has been demonstrated in the pharynx of both the free-living *Caenorhabditis elegans* (Zhang et al., 2005) and the gastrointestinal parasite *Oesophagostomum dentatum* (Neuhaus et al., 1997). There are two chitin synthase genes in *C. elegans*, *chs-1* and *chs-2*, encoding eggshell and pharynx chitin, respectively, and *chs-2* knockdown leads to lack of pharyngeal function and starvation (Zhang et al., 2005). This suggests that host chitotriosidase could potentially interfere with parasitic nematode feeding. Two studies to date have investigated associations between *CHIT1* genotype and filarial nematode infection. Choi et al. (2001) found significant association between susceptibility to lymphatic filariasis (*Wuchereria bancrofti*) and homozygosity for the mutant allele in an Indian population, but in a similar study in Papua New Guinea, no association between *CHIT1* genotype and lymphatic filariasis was observed (Hise et al., 2003).

There have been no previous studies investigating *CHIT1* genotype and gastrointestinal nematode infection. Human hookworm infection is an abundant chronic gastrointestinal nematode infection in sub-tropical and tropical countries, causing significant morbidity, principally due to iron-deficiency anaemia (Brooker et al., 2004). Human hookworm burden (as assessed by faecal egg count) is known to be under host genetic control (Williams-Blangero et al., 1997), but the genes responsible have not been identified. The aims of the current study were (1) to investigate associations between *CHIT1* genotype and human hookworm infection in an endemic population from Madang Province, Papua New Guinea and (2) to investigate evidence for directional selection at the *CHIT1* locus, by comparing worldwide allele frequency variation at the *CHIT1* locus to that observed at other loci across the genome.

2. Materials and methods

2.1. Geographical location and study population

The study population consisted of five villages in lowland Madang Province, Papua New Guinea, where *Necator americanus* is the only hookworm species (Pritchard et al., 1990). Villages were censused and pedigree information collected in 1998, and sample containers for faecal collection offered to all individuals aged 4 years and above. Faecal egg counts were performed using a modified McMaster salt flotation method and results were expressed as eggs per gram (epg) of faeces (Quinnell et al., 2004). Venous blood was then collected, and all individuals were offered treatment with albendazole or pyrantel pamoate. DNA was extracted from buffy coats using phenol/chloroform. In September 2001, a second faecal sample was taken from three of the five villages and reinfection hookworm burden determined; some previously untreated individuals provided samples in 2001. Hookworm epg has been shown to be a heritable phenotype in the study population (Breitling and Quinnell, unpublished results). The study was approved by the Medical Research Advisory Committee of Papua New Guinea and informed consent was received from all subjects or their parents.

2.2. Chitotriosidase genotyping by PCR

Thermal cycling reactions consisted of Reddy Mix PCR master mix (ABgene), 4.5 pmol forward and 4.5 pmol reverse chitotriosidase primers (forward: AGCTATCTGAAGCA-GAAG; reverse: GGAGAAGCCGGAAAGTC) (Sigma) and 2 μl genomic DNA in a final volume of 15 μl. Samples were run in 96 well PCR plates (ABgene) on a Peltier PTC-200 Thermal Cycler (MJ Research). The thermal cycling protocol was: 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 54 °C for 30 s and 72 °C for 30 s; and 72 °C for 5 min. Thermal cycling products with 10 base pair markers (Promega) were separated on 4% high resolution agarose (Sigma) gels in 1× TBE buffer and ethidium bromide. Gels were viewed using the Gene Genius Bio-imaging System and Gene Snap software (Syngene). The size of wild type product is 75 base pairs whereas the mutant product is 99 base pairs due to the mutant allele containing a 24 base pair duplication in exon 10 (Boot et al., 1998).

2.3. Statistical analysis

Variation in faecal egg counts by *CHIT1* genotype was analysed under both a codominant and recessive model. Faecal egg counts were highly overdispersed, so analysis was performed using a generalised linear model with a negative binomial error structure in Stata 9.1. This method has been shown to be more accurate than analysis of log-transformed parasite burden data (Wilson et al., 1996). Significant covariates included in the analysis were age, village, faecal consistency, anthelmintic treatment and the age × treatment and village × treatment interaction terms. Egg counts from both years were analysed together; since some individuals were sampled both pre- and post-treatment, standard errors were adjusted for the non-independence of samples from the same individual using the 'cluster' option. To control for non-independence of individuals due to genetic relatedness, further analysis was performed with the total test of association in a variance components framework using the programme QTDT (Abecasis et al., 2000). Finally, to control for potential population stratification, transmission disequilibrium testing was carried out using the orthogonal association model in QTDT. The phenotypic variable used for these analyses was the residuals from a negative binomial regression of egg counts against significant covariates; where two samples were taken from the same individual, the mean residual for that individual was calculated.