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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Vitamin D binding protein polymorphism protects against development of blastomycosis

Le polymorphisme de la protéine de liaison de la vitamine D protège conte la blastomycose

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Summary Blastomycosis is an uncommon endemic fungal infection. It is presumed that in the endemic regions, the number of exposed individuals is significantly greater than those in whom clinical manifestations develop. We conducted a case-control study of individuals with clinical blastomycosis and controls with similar exposure but who did not develop disease. A genetic association was observed between the Gc-2 allele of vitamin D binding protein and reduced susceptibility to blastomycosis in a Canadian cohort. The Gc-2 allele can affect increased antimicrobial activity of macrophages. It may be possible to mimic this mechanism of protection by vitamin D supplementation.

Résumé La blastomycose est une infection fongique endémique assez rare. En zone endémique, on estime que le nombre de personnes exposées est nettement plus élevé que celui des personnes qui ont des manifestations cliniques. Nous avons mené une étude cas—témoins chez

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les sujets avec une blastomycose et chez des témoins avec une exposition similaire mais qui n'ont pas développé la maladie. Une association génétique a été observée entre l'allèle Gc-2 de la protéine de liaison de la vitamine D et une diminution de la sensibilité à la blastomycose dans une cohorte canadienne. L'allèle Gc-2 peut concerner une activité anti-microbienne accrue des macrophages. Il pourrait être possible de reproduire ce mécanisme de protection par l'apport de vitamine D.

Introduction

Blastomycosis is an uncommon fungal infection caused by the organism *Blastomyces dermatitidis* endemic to portions of North America. Blastomycosis has a range of presentations, running the gamut from acute pneumonia syndromes to a chronic disease that mimics pulmonary tuberculosis, manifesting with symptoms of low grade fever, a productive cough, night sweats and weight loss. Extrapulmonary manifestations, involving bones, skin and central nervous system, and reactivation of the disease in the context of immune suppression are additional similarities [3,5]. Early histological reports of blastomycosis also reported the development of tubercle-like nodules in the lungs with multinuclear giants cells with very few features to distinguish from tuberculosis [4,10]. Also like tuberculosis, human populations show variable susceptibility to blastomycosis with clinical cases representing the proverbial ''tip of the iceberg'' in endemic areas where exposure is likely common. Even in regions with high endemicity of blastomycosis, often only one member of a family develops clinical manifestations, despite consistent environmental exposure. A recent report of a large outbreak from Wisconsin, included 55 cases in Wisconsin, includes clustering of over than half of the cases and highlights a significant increase in incidence since 2005 as well as higher rates among Asians than non-Asians (2010 incidence: 168 vs. 13 per 100 000 population), in the absence of difference in outdoor exposure [12]. The findings suggest a potential role for host genetic factors involvement in disease susceptibility. This apparent disparity in susceptibility to blastomycosis may be the result of an individual's ability to limit the progress of disease without need for treatment [9].

Variable bioavailability and metabolism of vitamin D has been associated with susceptibility to tuberculosis, in part, because vitamin D can modulate the function of activated macrophages [1]. The vitamin D derivative 25-hydroxyvitamin D3 (25-OHD3) is sequestered and transported by vitamin D binding protein (VDBP) to cells and tissues, although free form 25-OHD3 is reported to be more accessible to target cells with a corresponding higher biological response [14]. In macrophages, 25-OHD3 is converted to 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) which binds to vitamin D receptors b (VDR) in the cell. Binding to the receptor mediates the induction of the immunomodulatory peptide cathelicidin, subsequently fostering antimicrobial activity against pathogens. Genetic polymorphisms in VDBP and VDR can affect this process and have been associated with susceptibility to tuberculosis and other infectious diseases (e. g. [8,11]).

Observing the clinical parallels between tuberculosis and blastomycosis raises the question of similar susceptibility factors for these two diseases. Thus, the aim of this study was to genotype two single nucleotide polymorphisms (SNP) in the VDR gene and one SNP in the VDBP gene, with correlations to tuberculosis susceptibility, and test for associations in a blastomycosis case-control cohort contributing to a better understanding of this unusual disease.

Methods

Study participants

This study was approved by the Human Research Ethics Board of the University of Manitoba. After written informed consent was obtained, whole blood from patients diagnosed with blastomycosis ($n = 28$) was obtained. The samples were spun down, buffy coat separated, and frozen for later analysis. DNA was extracted from whole blood using a DNeasy Blood (Qiagen Inc. Toronto, ON), following manufacturer's instructions.

Genotyping

Following the methods employed by Larcombe et al. [6], three SNPs were genotyped. In the VDR, the rs2228570 (T/C which creates the endonuclease site *Fok I* and is named accordingly with *F* [common] and *f* [minor] alleles), and rs1544410 (T/C which creates the endonuclease site *Bsm I* and is named accordingly with *B* [common] and *b* [minor] alleles) SNPs were genotyped. In the VDBP, rs4588 (C/A which results in an amino acid change from Threonine [T—common allele] to Lysine [K—minor allele] at position 436 in the translated protein) were genotyped. The choice of SNPs selected to be analysed was based on the fact that these are the three most common variants of VDBP and on their impact on VDBP protein structure, bioavailability of VDBP and its binding affinity to 25-OHD3. The following PCR conditions were used for VDBP amplification in a 25 μl reaction: 3.5 μl extracted DNA; 2.5 μl 10X PCR Buffer; 1.0 μl 50 mM $MgSO_4$; 0.2 μl 10 mM dNTP mix; 16.2 μl reagent grade water; 0.1 μl Platinum® Taq (Invitrogen Life Technologies Corp. Burlington, ON), and 0.5 μl each of the forward primer and reverse primers. PCR conditions were 95 °C for 15 min followed by 35 cycles of 94 °C for 20 s, 58 °C for 20 s, and 72 °C for 20 s with a 1 s increment for each subsequent cycle, and one cycle at 72 °C for 10 min, as described in [7].

Statistical analysis

GraphPad Prism 4 (GraphPad Software, San Diego) was used to test for deviation from Hardy-Weinberg Equilibrium (HWE)