Short communication

NINJURIN1 single nucleotide polymorphism and nerve damage in leprosy


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Leprosy, a chronic infectious disease caused by Mycobacterium leprae, can damage the peripheral nervous system and represents one of the leading causes of nontraumatic neuropathy in some developing countries. The NINJURIN1 is a cell adhesion molecule that provides suitable substrates for repair of Schwann cells after peripheral nerve injury. The single nucleotide polymorphism NINJ1, is the result of a transversion of an adenine to a nucleotide polymorphic cytokine (A → C), responsible for an amino acid exchange of asparagine to alanine at position 110 of the protein (asp110ala).

Objectives: The aim of this study was to investigate the importance of the polymorphism in the NINJ1 gene for neural impairment during leprosy course.

Methods: A single nucleotide polymorphism (asp110ala) was searched in 218 leprosy patients and 244 non-leprosy subjects using a polymerase chain reaction/restriction fragment length polymorphism (PCR–RFLP) method.

Results: No statistical differences were observed in the frequency of the asp110ala SNP between leprosy patients versus non-leprosy and multibacillary versus paucibacillary clinical forms. The C allele (ala110) is increased among patients exhibiting nerve impairment (p = 0.0379). Also, leprosy patients with the CC genotype (ala/ala) had a higher risk (OR = 4.21) of developing nerve disability when compared those carrying the AA genotype (asp/asp) (OR = 0.69).

Conclusion: Our results show an association between the studied C allele (ala110) and damage nerve in leprosy patients.

Significance: Ninjurin analysis showed that asp110ala could be a valuable prognostic marker, since C allele (ala110) have increased susceptibility to nerve damage.

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1. Introduction

Hansen’s disease, or leprosy, is a chronic infectious disease caused by Mycobacterium leprae, an obligate intracellular acid-fast bacillus. Leprosy is one of the leading causes of nontraumatic neuropathy in developing countries and an example of peripheral nervous system (PNS) infectious neurodegenerative disease (Rambukkana, 2010; Ooi and Srinivasan, 2004). In spite of the improving results from multidrug therapy (MDT), in the last two decades leprosy still remains an important global health problem (World Health Organization, 2001); mainly because of disabilities and deformities caused by peripheral neuropathy (Agrawal et al., 2005).

The M. leprae parasites macrophages and Schwann cells of the peripheral nerves. In addition, M. leprae has the capacity to pass over the Schwann cells basal lamina and, thereafter, remains secure from antimicrobial drugs. This allows the bacillus to multiply continuously, causing nerve injury (Rambukkana, 2010). The nerve damage affects sensory, motor, and autonomic fibers and occurs gradually throughout the course of the disease. The affinity of M. leprae for Schwann cells is mediated by α2-laminin, a major component of the basal lamina, which binds α-dystroglican in the cell membrane (Smith et al., 2009).

Clinically, leprosy is classified according to the number of skin lesions and quantity of bacilli in those lesions. Ridley–Jopling classification (Ridley and Jopling, 1966), the most used grading for leprosy, is based on clinical, bacteriological, immunological and histopathological features. Tuberculoid leprosy (TT), is characterized by isolated skin lesions without or rare intracellular bacilli and T helper 1 (Th1) cellular immune responses. Lepromatous leprosy (LL), on opposite to TT, is characterized by the presence of...
proliferate bacilli in the skin and T helper 2 (Th2) humoral immune responses. Three intermediate forms, borderline tuberculoid (BT), borderline borderline (BB), borderline lepromatous (BL) and indeterminate (I), are also observed. Leprosy patients can also develop two types of reaction: type 1 or reversal reaction (RR); and type 2 reaction or erythema nodosum lepromatous (ENL). Both are characterized by the reactivation of the host immune response and often exacerbate nerve injury (Berrington et al., 2010).

Studies have demonstrated that the binding of \textit{M. leprae} to Schwann cells can induce both demyelination and axonal damage. Schwann cells play an important role in regeneration after neuronal damage (Rambukkana, 2001).

The influence of individual genetics factors leading to different immune responses is clearly observed for leprosy susceptibility, as well as for its clinical course and type of disease; moreover, specific molecular variants could be important to define increased individual risk for disease after exposition to \textit{M. leprae} (Cardoso et al., 2007; Schurr et al., 2007). Nevertheless, genetic studies to elucidate the mechanism of peripheral nerve damage have not been accomplished.

Ninjurin (nerve injury-induced protein) is a cell adhesion molecule that provides suitable substrates for repair of Schwann cells after peripheral nerve injury. The Ninjurin gene (NINJ1), which encodes the protein Ninjurin1, is located in chromosome 9q22.A non-synonymous A–C transversion in its third exon results in amino acid change at position 110 (asp110ala) of the protein (Cardoso et al., 2007; Araki and Milbrandt, 1996; Kim et al., 2001).

In the present study we hypothesized that the presence of polymorphism in the NINJ1 gene could be relevant for neural impairment during the course of leprosy.

The aim of this study was to evaluate the importance of the NINJ1 gene single nucleotide polymorphism (SNP) on leprosy susceptibility modulation and/or to modify its clinical outcome, either PB or MB, in a large cohort of patients. We also studied the correlation between this polymorphism and nerve injury.

2. Materials and methods

2.1. Study setting and population

The study population was composed by individuals older than 18 years old, belonging to all ethnic groups, invited to participate after signing an informed consent. This study was approved by the Ethical Research Board from the Faculdade de Medicina de São José do Rio Preto – FAMERP (SISNEP n° 0036.0.140.000-06).

2.1.1. Patients

This study represents a case-control investigation of 218 leprosy patients \((n = 218)\), 103 female and 115 male, recruited from two leprosy treatment reference centers in São José do Rio Preto city, located in the Northwestern region of São Paulo state, Southeast Brazil: the Núcleo de Gestão Assistencial 60 (NGA-60) and the Regional Center of Leprosy Diagnosis (NGA 60), the regional center of leprosy diagnosis and treatment. Together, these two centers concentrate leprosy patients (SISNEP n° 0036.0.140.000-06).

Analyses of genotypic and allelic frequencies of asp110ala SNPs in patients versus controls; MB versus PB and DG = 0 versus DG > 0 were carried out using Chi-square test by software Minitab (Version 15). Odds ratios and 95% CL adjusted for age and genders were then estimated using logistic regression analysis as an estimate of the relative risk and strength of association. A \(P\)-value < 0.05 was accepted as statistically significant.

3. Results

The mean ages of patients and controls (±SD) were 55 ± 14 and 51.4 ± 16 years, respectively. There were no statistically significant differences between cases and controls (age and ethnicity), indicating a well-matched population. The predominance of the cases tuberculoid leprosy in the study region in relation to borderline tuberculoid cases is an important epidemiological pointer of the growing tendencies of the disease, demonstrating an involvement of the population able to develop more intense cellular immunity against \textit{M. leprae} and carrier of natural resistance to this bacillus (Table 1). The genotypic frequencies of asp110ala SNP were classified according to the Ridley Jopling Scale (Ridley and Jopling, 1966) and also as paucibacillary (PB) or multibacillary (MB), with subsequent treatment according to World Health Organization (WHO) specifications (World Health Organization, 1998). The PB group (BT, TT and I) was constituted by 96 cases, while the MB group (LL, BL and BB) was constituted by 122 cases. The disability grade was assessed according to WHO (Pimentel et al., 2004); and is performed routinely in all recently diagnosed leprosy patients. Eyes, hands and feet were analyzed and each received a grade ranging from 0 to 2 at diagnosis. Grade 0 means no observation of disability due to leprosy while grade 1 show sensory nerve impairment. Grade 2 also show motor nerve involvement (Pimentel et al., 2004; Ministério da Saúde, 2008). Either grade 1 or 2 were grouped considered to the disability grade (DG) of the patient. Patients were classified as paucibacillary (PB) or multibacillary (MB) and treated according to WHO specifications. Clinical classification and disability grade (DG) of leprosy patients group are shown in Table 1.

2.1.2. Controls

Two-hundred and forty four \((n = 244)\) unrelated individuals, 110 female and 134 male, from the same endemic area as the patients were selected among blood bank donors from the HB Blood Bank, in the same municipality, and were included as controls. They were matched with leprosy patients by age (±5 years) and gender. None of them referred disease or were taking continuous medication.

2.2. Genomic DNA extraction

The genomic DNA was obtained from peripheral blood according to modified Miller et al. (1988); technique. The NINJ1 gene polymorphism was investigated by polymerase chain reaction with restriction fragment length polymorphism (PCR–RFLP), according to the method described by Cardoso et al. (2007), the primers and enzyme used were: sense 5'-CTC ATC C-3' (NINJ1F) and anti-sense 5'-CCT CGCGCCCCATCTCC G-3' (NINJ1R) and HAE III (New England Biolabs, Beverly, MA, USA). A HaeIII cleavage site is generated when the C allele (ala110) is present, in such way that the genotypes were clearly ascertained by the size of the fragments (212 bp for the A allele; 176 bp for the C allele).

2.3. Statistical analysis

The mean ages of patients and controls (±SD) were 55 ± 14 and 51.4 ± 16 years, respectively. There were no statistically significant differences between cases and controls (age and ethnicity), indicating a well-matched population. The predominance of the cases tuberculoid leprosy in the study region in relation to borderline tuberculoid cases is an important epidemiological pointer of the growing tendencies of the disease, demonstrating an involvement of the population able to develop more intense cellular immunity against \textit{M. leprae} and carrier of natural resistance to this bacillus (Table 1).
initially compared between leprosy patients and the control group, using the variables gender and age in the logistic regression model, and no statistically significant differences was observed. Also, no differences were observed in the frequency of asp110ala SNP between MB and PB groups (Table 2).

The asp110ala SNP versus leprosy neural damage, measured herein by the disability grade (DG), was evaluated in leprosy patients. For statistical analysis, we only considered absence (DG = 0) or presence (DG > 0) of initial (at the time of diagnosis of leprosy) disability grade. The frequency of allele A (asp110) was 0.561 in patients with disability grade (DG > 0) and 0.803 in patients absence of disability grade (DG = 0). And the allele C (ala110) was 0.225 in patients DG > 0 and 0.196 in patients DG = 0. The allelic frequencies of allele C (ala110) was found at increased frequency in leprosy patients with sensory or motor nerve impairment (DG > 0) compared with patients showing no disability due to leprosy (DG = 0) (p = 0.037). Furthermore leprosy patients with the CC polymorphic genotype (ala/ala) had a higher risk (OR = 4.21) of developing nerve disability when compared those carrying the AA genotype (asp/asp) (OR = 0.69) (Table 3).

4. Discussion

The NINJ1 gene plays an important role in nerve regeneration. The ninjurin protein is transported down the length of the axon to the site of injury, where it accumulates (Lee et al., 2010). Therefore, polymorphism in this gene could be related to nerve damage in leprosy patients. In this study we investigated the role of this gene with leprosy occurrence and neural impairment during the course of disease.

Our results did not find positive association of this SNP to leprosy or even to its clinical subtypes. However, we found statistically significant association for DG leprosy patients. Patients with C allele (ala110) were more susceptible to develop nerve damage, assessed by DG.

To our knowledge, this study is the second to associate polymorphisms in the NINJ1 gene with leprosy nerve damage. Cardoso et al. (2007) were the first to study this polymorphism and its relationship with protection in leprosy nerve damage. They also found a significant association between the C allele and nerve damage in leprosy patients.

One possible fail in our study was the lack of electrophysiological evaluation that could be useful to add more information beyond clinical disability in order to evaluate nerve damage. However, this fact did not strongly modify our results, since nerve damage mostly is clinically evident.

As ninjurin is induced by nerve injury by leprosy and have a strong role for neuronal recovery by increasing cell adhesion and Schwann cells regeneration (Lee et al., 2010); its presence could be relevant for leprosy recovery after nerve damage. NINJ1 is also associated with several diseases, e.g., after spinal cord injury (Araki and Milbrandt, 1996); where it is up-regulated in Schwann cells and dorsal root ganglion neurons, acute lymphoblastic leukemia (Chen et al., 2001); in B cells, and in multiple sclerosis (Ahn et al., 2009; Tajouri et al., 2007). These studies indicate that ninjurin is an important adhesion molecule and could also be participating in many neurodegenerative diseases.

The results of this study reinforce previous findings (Cardoso et al., 2007) on this polymorphism association with nerve damage in leprosy patients. In addition, in our study population carriers of the CC genotype (ala/ala) had a higher risk (ORs) of developing disability. Together, these data encourage future efforts towards the establishment of a NINJ asp110ala genotyping as a prognostic marker of nerve damage that could lead to a preventive corticosteroid treatment protocol. In conclusion, the possibility of using a polymorphism, which is easily determined by minimal invasive methods, as a prognostic marker, opens new perspectives on the approaches to be used in the clinic, and the possibility of identifying patients at high risk for the development of more aggressive disease.

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References