Susceptibility to non-tuberculous mycobacterial disease is influenced by rs1518111 in *IL10*

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Keywords

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Abbreviations

NTM: Nontuberculous Mycobacteria, MA: Minor Allele, MAF: Minor Allele Frequency, IL: Interleukin, SNP: single nucleotide polymorphism, COPD: chronic obstructive pulmonary disease, HWE: Hardy-Weinberg Equilibrium

Abstract

Although exposure to potentially pathogenic nontuberculous mycobacteria (NTM) via soil and domestic water supplies is common, pulmonary infection and disease are confined to a small proportion of older individuals. We associated alleles of a polymorphism in *IL10* (rs1800896) with NTM disease and demonstrated elevated production of IL-10 by blood leukocytes from patients with pulmonary NTM. Here we investigate seven additional polymorphisms in *IL10* in a larger cohort of Caucasian controls and patients with pulmonary NTM disease. We describe a significant association between pulmonary NTM disease and one polymorphism (rs1518111) in strong linkage disequilibrium with rs1800896.

Introduction

Pulmonary disease is the most common presentation of infections with non-tuberculous mycobacteria (NTM) in otherwise healthy adults, and is considered an emerging health risk in Australia and around the world [1, 2]. Exposure through soil or water is common, but few people develop pulmonary NTM disease. Estimates from Queensland, Australia, suggest a frequency of 22.1 persons per 100 000 in the population [1]. Known risk factors for disease include smoking, excessive alcohol intake, past tuberculosis and underlying lung diseases such as COPD and bronchiectasis [2, 3]. However these factors do not account for all cases – a notable exception being patients with the "Lady Windermere" phenotype. These individuals are lean and tall, with higher than normal incidence of thoracic abnormalities including scoliosis, pectus excavatum and mitral valve prolapse. The majority of pulmonary NTM cases are Caucasian (91%) and female (95%), with an average age of 60 years [4]. Behavioural questionnaires have found no links between pulmonary NTM disease and patients' activities, exposures or habits [4]. This implicates genetic factors in susceptibility and disease severity.

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Effective Th1 and Th17 cell-mediated immune responses are crucial for the clearance of NTM infection and can be inhibited by IL-10 produced by Th2 cells and macrophage. Hence polymorphic alleles affecting either the expression or activity of *IL10* may impact upon the Th1/Th2/Th17 balance/response in patients of pulmonary NTM disease. Our studies of cytokine production by blood leukocytes from patients with pulmonary NTM disease demonstrated comparatively high IL-10 responses [5]. Interferon-γ/IL-10 ratios are also associated with disease severity in tuberculosis, supporting a role for IL-10 in mycobacterial immunity [6].

Previously, we and others have linked differences in allele frequencies for single nucleotide polymorphisms (SNP) within the *IL10* promoter with pulmonary NTM disease (rs1800896), asthma and tuberculosis (rs1800896, rs1800871, and rs1800872) [7-10]. To further understand the influence of genetics on disease, we typed seven additional SNP within *IL10* in an expanded cohort of patients with pulmonary NTM and healthy controls. SNP were chosen on the basis of previous publications suggesting a role in inflammatory disease &/or changing the encoded amino acid &/or location in a putative regulatory region. SNP that were monomorphic in Asians, Africans or Caucasians were excluded.

Materials and Methods

Patients and controls

DNA was obtained from 124 patients with pulmonary NTM disease attending Greenslopes Private Hospital, QLD, Australia and Prince Charles Hospital, QLD, Australia between 2005 and 2014. NTM infection was diagnosed according to American Thoracic Society criteria [11] using radiological and microbiological findings. All participants provided written informed consent and the project was approved by the Greenslopes Research and Ethics Committee (Protocol 12/12) in accordance with

the National Statement on Ethical Conduct in Human Research. Two hundred and twenty nine control donors were recruited from Western Australia[12] and DNA samples were provided by the Institute for Respiratory Health, Western Australia, in accordance with a Royal Perth Hospital Human Research Ethics Approval. The cohort is considered to be representative of the Caucasian Australian population from which patients were also derived.

Genotyping

DNA samples were quantified by fluorometry with a Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) and adjusted to 50ng/uL. Genotyping was performed using custom TaqMan OpenArray Genotyping Plates (Life Technologies, Grand Island, NY, USA) [13]. DNA samples were diluted at 1:1 in TaqMan OpenArray Genotyping Master Mix for 50 cycles of PCR amplification. The output was viewed using OpenArray™ SNP Genotyping Analysis software, and genotypes were allocated manually. The SNP rs3024497 was excluded as it did not meet Hardy-Weinberg Equilibrium (HWE). The genotyping success rate for the remaining SNP averaged 94.4 %.

Data analysis

Linkage between paired SNP alleles was determined with the use of Ensembl's "Pairwise linkage disequilibrium data by population" tool with the 1000 genomes database. In this way, linkage is determined via both R^2 and D` values for each ethnicity with available data. Statistical analyses were performed in Stata 12 (StataCorp, Collage Station, TX, USA). Univariate analyses evaluating associations between pulmonary NTM lung disease and gender, age, SNP and haplotypes were performed using two-tailed Fisher's exact or Chi^2 tests (χ^2) as appropriate. Multivariate analyses were performed using logistic regression modelling using all included factors associated with NTM in univariate analyses ($p < 0.20$ cut-off) followed by a stepwise removal procedure to obtain the model of best fit.

Results

The demographic profile of patients with pulmonary NTM disease

We studied 124 patients with pulmonary NTM disease and 229 healthy controls of Caucasian ethnicity. Seventy-three percent of patients and 55% of healthy controls were female. As this was a statistically significant difference (χ^2, p = 0.001), gender was included in logistic regression models with SNPs of interest.

NTM patients were older than healthy controls [median (range) 67 (25-89) vs. 44 (21-75) years, respectively]. Whilst some control donors may develop pulmonary NTM lung disease later in life, the impact on the data is likely to be minor as pulmonary NTM disease is rare. Patients were infected with *M. intracellulare* (n = 64), *M. avium* (n = 13), *M. abscessus* (n = 10), *M. kansasii* (n = 3), *M. triplex* (n = 2), *M. xenopi* (n = 1), *M. simiae* (n = 1), *M. interjectum* (n = 1), *M. shimoidei* (n = 1), *M. terrae* (n = 1), *M. gordonae* (n = 1), or *M. lentiflavum* (n = 1). Some individuals were infected with more than one mycobacterial species (n = 12).

Associations between SNP genotypes and pulmonary NTM disease

Of the seven SNP analysed, rs1518111 was the only marker showing a statistically significant association with pulmonary NTM disease (*P =0.004*) in univariate χ^2 analysis. This withstood Bonferroni correction for multiple comparisons (Table 1). rs03024498 and rs1800872 displayed weaker associations with disease (χ^2, $P < 0.06$). These three SNP were included in logistic regression models with gender. After a stepwise removal process, the final model (model *p < 0001; R² = 0.05*) included only gender and rs1518111 (Table 2). When gender was not included, the model was

weakened but remained comparable, demonstrating the associations between the minor alleles of rs1518111 and rs3024498 with reduced and increased risk of disease (Table 2).

We assessed whether radiological findings (nodular bronchiectasis, cavitary, or mixed) were associated with alleles of the rs1518111 SNP. Cavitary disease was more common in patients carrying the minor allele (A) at rs1518111 (χ^2, $P = 0.001$). The isolation of fast (n=9) or slow (n=86) growing mycobacteria from the lung were also tested for associations with alleles of rs1518111. A weak association was observed between fast growing NTM species and carriage of the minor allele (χ^2, $P = 0.07$).

Discussion

The minor (A) allele of rs1518111 (previously denoted rs3748675) was associated with reduced risk of pulmonary NTM disease. This minor (A) allele has been linked to increased incidence of Behcet's disease in a Turkish cohort [14], reduced risk of prostate hyperplasia in a Korean cohort [15], and increased risk of tuberculosis (as part of a three SNP haplotype) in an Ugandan cohort [16]. The allele is also associated with lower IL-10 plasma concentrations, poor treatment outcome and enhanced systemic inflammation in patients with acute coronary syndrome in a Swedish cohort [17]. Studies investigating this allele as a contributing factor to disease identify associations with pro-inflammatory tendencies in patients, suggesting a modified IL-10 response. Our study implicates the minor (A) allele of rs1518111 in risk of pulmonary NTM disease in Caucasians.

Ethnicity is an important consideration because the frequency of the A allele of rs1518111 varies between different ethnicities, from 22% in Europeans (EUR) to 68% in East Asians (EAS) [18]. rs1518111 is in linkage disequilibrium with rs1800896 in all ethnicities covered by the 1000 Genomes database

(D' > 0.99) [18]. However R^2 values range from 0.095 (Chinese Dai in Xishuangbanna, China) to 0.420 (Mandinka, Western Gambia). The minor (C) allele of rs1800896 is usually inherited with the minor (A) allele of rs1518111, but rs1800896 has a higher MAF. Both alleles have now been associated with NTM disease [7], demonstrating the existence of an *IL10* haplotype which could affect the ability of an individual to respond effectively to pulmonary NTM infection. In individuals from the control population used in our study, a haplotype spanning the promotor region of *IL10* affected transcription regulation in peripheral blood cells stimulated with *Streptococcus pneumonia* [12]. The two SNP associated here with NTM disease (rs1800896 and rs1800872) were in complete linkage disequilibrium [12]. In South African HIV patients, we have associated rs1800896 [19] and rs1518111 with peripheral neuropathy (Goullee et al unpublished data), providing another link between these alleles and inflammatory disease. Unfortunately the linkage disequilibrium confounds determinations of which SNP affects IL-10 levels *in vivo.*

Radiological data was available for a subset of the patients used in this study. Amongst these, carriage of the minor (A) allele at rs1518111 correlated with a presentation of cavitary disease (as opposed to nodular bronchiectasis) so the immunopathology of these presentations may vary. Infection with fast and slow growing NTM species is independent of radiological presentation and only weakly associated with the *IL10* genotype.

In conclusion, when compared with a healthy control group, patients with pulmonary NTM disease were less likely to carry the minor (A) allele for the *IL10* SNP rs1518111, which is in linkage disequilibrium with rs1800896 – a SNP previously associated with NTM disease. Our review of the literature associates these alleles with low levels of IL-10 and enhanced risk of inflammatory conditions.

References

1.   Thomson RM. Changing epidemiology of pulmonary nontuberculous mycobacteria infections. *Emerging infectious diseases* 2010; **16**(10)**:** 1576-1584.

2.   Winthrop KL, McNelley E, Kendall B, Marshall-Olson A, Morris C, Cassidy M *et al.* Pulmonary nontuberculous mycobacterial disease prevalence and clinical features: an emerging public health disease. *American journal of respiratory and critical care medicine* 2010; **182**(7)**:** 977-982.

3.   Cassidy PM, Hedberg K, Saulson A, McNelly E, Winthrop KL. Nontuberculous mycobacterial disease prevalence and risk factors: a changing epidemiology. *Clinical Infectious Diseases* 2009; **49**(12)**:** e124-e129.

4.   Kim RD, Greenberg DE, Ehrmantraut ME, Guide SV, Ding L, Shea Y *et al.* Pulmonary nontuberculous mycobacterial disease: prospective study of a distinct preexisting syndrome. *American journal of respiratory and critical care medicine* 2008; **178**(10)**:** 1066-1074.

5.   Lim A, Allison C, Price P, Waterer G. Susceptibility to pulmonary disease due to Mycobacterium avium–intracellulare complex may reflect low IL-17 and high IL-10 responses rather than Th1 deficiency. *Clinical Immunology* 2010; **137**(2)**:** 296-302.

6.   Jamil B, Shahid F, Hasan Z, Nasir N, Razzaki T, Dawood G *et al.* Interferonγ/IL10 ratio defines the disease severity in pulmonary and extra pulmonary tuberculosis. *Tuberculosis* 2007; **87**(4)**:** 279-287.

7.   Affandi JS, Hendry S, Waterer G, Thomson R, Wallace H, Burrows S *et al.* Searching for an immunogenetic factor that will illuminate susceptibility to non-tuberculous mycobacterial disease. *Human immunology* 2013; **74**(10)**:** 1382-1385.

8.   Gao X, Chen J, Tong Z, Yang G, Yao Y, Xu F *et al.* Interleukin-10 promoter gene polymorphisms and susceptibility to tuberculosis: a meta-analysis. *PloS one* 2015; **10**(6)**:** e0127496.

9.   Hsia T-C, Chang W-S, Wang S, Shen T-C, Hsiao W-Y, Liu C-J *et al.* The Contribution of Interleukin-10 Promoter Genotypes to Susceptibility to Asthma in Adults. *In Vivo* 2015; **29**(6)**:** 695-699.

10.   Liu Q, Li W, Li D, Feng Y, Tao C. The association of interleukin-10-1082,-819,-592 polymorphisms and tuberculosis risk. *Saudi Medical Journal* 2015; **36**(4)**:** 407.

11.   Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F *et al.* An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *American journal of respiratory and critical care medicine* 2007; **175**(4)**:** 367-416.

12.   Temple SE, Lim E, Cheong KY, Almeida C-AM, Price P, Ardlie KG *et al.* Alleles carried at positions− 819 and− 592 of the IL10 promoter affect transcription following stimulation of peripheral blood cells with Streptococcus pneumoniae. *Immunogenetics* 2003; **55**(9)**:** 629-632.

13.   Goullee H, Wadley AL, Cherry CL, Allcock RJ, Black M, Kamerman PR *et al.* Polymorphisms in CAMKK2 may predict sensory neuropathy in African HIV patients. *Journal of neurovirology* 2016**:** 1-10.

14.   Remmers EF, Cosan F, Kirino Y, Ombrello MJ, Abaci N, Satorius C *et al.* Genome-wide association study identifies variants in the MHC class I, IL10, and IL23R-IL12RB2 regions associated with Behçet's disease. *Nature genetics* 2010; **42**(8)**:** 698-702.

15.   Yoo KH, Kim SK, Chung J-H, Chang S-G. Association of IL10, IL10RA, and IL10RB polymorphisms with benign prostate hyperplasia in Korean population. *Journal of Korean medical science* 2011; **26**(5)**:** 659-664.

16.   Stein CM, Zalwango S, Chiunda AB, Millard C, Leontiev DV, Horvath AL *et al.* Linkage and association analysis of candidate genes for TB and TNFα cytokine expression: evidence for association with IFNGR1, IL-10, and TNF receptor 1 genes. *Human genetics* 2007; **121**(6)**:** 663-673.

17.   Mälarstig A, Eriksson P, Hamsten A, Lindahl B, Wallentin L, Siegbahn A. Raised interleukin-10 is an indicator of poor outcome and enhanced systemic inflammation in patients with acute coronary syndrome. *Heart* 2008; **94**(6)**:** 724-729.

18.   Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, *et al* with the 1000 Genomes Project Consortium. A global reference for human genetic variation. *Nature* 2015; **526**(7571)**:** 68-74.

19.   Wadley A, Kamerman P, Chew C, Lombard Z, Cherry C, Price P. A polymorphism in IL4 may associate with sensory neuropathy in African HIV patients. *Molecular immunology* 2013; **55**(3)**:** 197-199.

Table 1: rs1518111 was significantly associated with NTM disease in univariate analyses

SNP [a]	Chr. 1 Position	MA	MAF [b] Control	MAF [b] NTM	P-value (χ^2)
			(n = 229)	(n = 124)	
rs3024498	206941529	G	43.7%	54.9%	0.054
rs1518111	**206944645**	**A**	**43.7%**	**27.6%**	**0.004**
rs3021094	206944952	C	15.7%	18.5%	0.52
rs3024491	206945046	G	27.0%	22.1%	0.32
rs1800872	206946407	A	46.4%	35.8%	0.058
rs1800871	206946634	T	46.6%	38.5%	0.15

[a] SNP are shown in chromosomal order.

[b] Minor allele frequencies are based on individuals successfully genotyped

Table 2: Logistic regression models define gender and rs1518111 predict NTM disease.

Variable	Odds Ratio	P-value	95% confidence interval
Model combining gender and SNP (n = 338 [a], p < 0.0001; R^2 = 0.05)			
Gender	0.44	0.001	0.266-0.718
rs1518111	0.507	0.007	0.309-0.830
Model combining SNP (n = 315 [a], p = 0.0087; R^2 = 0.02)			
rs3024498	1.37	0.205	0.840-2.251
rs1518111 [b]	0.540	0.021	0.320-0.910

[a] excluding samples with genotyping failures.