Author’s response to reviews

Title: MiR-146a G/C rs2910164 variations in South African Indian and Caucasian patients with psoriatic arthritis

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Author’s response to reviews:

Response to Editor Comments:

Dear Editor

Thank you for the feedback and comments.

1. As both reviewers pointed out, the fact that the study is severely underpowered and no validation is presented is the main limitation of the study. This issue should be discussed in the manuscript.
Abstract:

Conclusion: lines 58-60:

The main limitation in this study was the small sample size in the case-control cohorts, with a low overall statistical power (post-hoc power analysis = 19%).

Introduction:

Lines 97-99:

This was done by comparing the rs2910164 genotype and allele frequency distribution between the Caucasian and Indian PsA patients and healthy controls for any similarities or deviations.

Materials and Methods:

Lines 104-106:

Blood samples were taken from South African Indian (n = 84) and Caucasian (n = 32) PsA patients (total n = 116) and healthy control subjects (Indian: n = 62 and Caucasian: n = 38; total n = 100)…

Lines 165-166

The post-hoc power analysis was used to calculate the overall statistical power of the present study [21, 22].
Results:

Lines 178-181:

The overall statistical power of this study (based on the post-hoc power analysis) was low (19%), however the sample size was adequate to compare the miR-146a G/C rs2910164 genotype and allele frequency distribution between the Caucasian and Indian PsA patients and healthy controls for any similarities or deviations.

Discussion:

Lines 221-285:

Data suggests that Indian PsA patients with the heterozygous GC and homozygous variant CC genotypes (GC+CC) are more predisposed to developing PsA compared to patients with the homozygous wild-type GG genotype.

Patients with uncontrolled psoriasis and PsA have exacerbated CRP levels [23, 24]. CRP is a biomarker of inflammation and disease severity. Psoriasis, PsA and rheumatoid arthritis are characterised by the secretion of several pro-inflammatory cytokines such as IL-2, IL-6, IL-8, IFN-γ and TNF-α. These cytokines are responsible for producing high levels of CRP and contributing to the pathophysiology of the disease. TNF-α induced secretion of IL-6 activates the production of CRP by stimulating the transcription of the CRP gene via activation of the IRAK1 and signal transducer and activator of transcription 3 (STAT3) inflammatory pathways; thereby, causing the high CRP levels observed in psoriasis, PsA and rheumatoid arthritis [25-27]. The PsA patients recruited in this study had high CRP levels (Table 1).

When miR-146a is highly expressed, it inhibits both IRAK1 and TRAF6 resulting in concomitant reductions in pro-inflammatory cytokines (IL-2, IL-6, IL-8, IFN-γ and TNF-α) expression and CRP levels [14]. The rs2910164 variant C-allele dampens the overall
functionality of miR-146a, leading to an upregulation in IRAK1 and TRAF6 expression, resulting in very high cytokine production [13].

In an Egyptian cohort, patients with psoriasis had abnormally higher miR-146a expression, and MTX treatment significantly reduced miR-146a expression [28]. Over 96% of PsA patients in our study received MTX that resulted in a significant decrease in CRP levels (CRP monitored at inclusion versus after 6 months) after treatment (p = 0.0011) (Table 1).

In 2014, Zhang et al. reported that Chinese patients homozygous for the wild-type GG genotype and heterozygous for the GC genotypes (GG+GC) compared to patients homozygous for the variant CC genotype had a greater risk of developing psoriasis and PsA [17]. The frequency of the wild-type G-allele was more common in psoriasis patients compared to healthy controls (48.2% versus 42.4%; p = 0.007). The frequency of the GG (21.7%) and GC (53.0%) genotypes were predominant among psoriasis patients versus controls (GG: 16.7% and GC: 51.5%) whereas the CC genotype was more common in the healthy controls versus psoriasis patients (31.8% versus 25.3%). The difference in the distribution of the rs2910164 genotypes between psoriasis patients and controls were statistically significant (p = 0.021). The combined frequency of the GG + GC genotypes were significantly higher among PsA patients compared to the controls (74.7% versus 68.2%; p = 0.018), and was associated with an increase in PsA susceptibility (adjusted OR = 1.38 95% CI 1.06–1.80) [17]. Chatzikyriakidou et al. (2010) found the frequency of the GC genotype to be higher in Greek PsA patients compared to healthy controls (41.4% versus 27.3%). The GG and CC genotypes were higher in the controls compared to PsA patients (GG: 59.1% versus 48.3% and CC: 13.6% versus 10.3%). However, no significant difference was observed in the distribution of the rs2910164 genotypes between PsA patients and controls (p = 0.394) [18].

In our study, the frequency of the GC+CC genotypes and variant C-allele were significantly higher in all PsA patients versus all healthy controls, with no significant changes in genotype distribution between patients and controls. No association between rs2910164 and PsA were noted in the Caucasian population. There was a significant difference in the distribution of the
rs2910164 genotypes between Indian PsA patients and controls. Indian PsA patients had a significantly higher frequency of the GC+CC genotypes and variant C-allele versus healthy controls (Table 3).

Psoriasis and PsA patients have a higher prevalence of MetS [29]. In 2012, Langhan et al. reported that patients with mild, moderate and severe psoriasis and PsA had a 22%, 56% and 98% chance of developing MetS, respectively [30]. PsA patients recruited in our study had a mean HAQ score indicative of moderate to severe functional impairments from the disease, and displayed increased fasting plasma glucose and HbA1c levels indicative of early stages of prediabetes and impaired fasting glucose (Table 1). Smoking, diet and obesity are some of the environmental risk factors associated with psoriasis and PsA [1]. Cigarette smoke affects the central nervous system and immune system, and causes pro-inflammatory and anti-inflammatory cytokines to be aberrantly expressed [1]. Nicotine binds to the nicotinic acetylcholine receptors (nAChR) found in several cell types, including immune B-cells, T-cells, thymocytes and leukaemic cell lines. This can lead to defective immune and nervous system signalling processes, and can negatively regulate keratinocyte function [1, 31]. Apart from psoriasis and PsA, smoking can also play an insidious role in triggering MetS [32]. In our study, PsA patients had a mean BMI indicating overweight and obesity, and over 21% were active smokers (Table 1).

Conclusion:

Lines 289-293:

This study associated the rs2910164 with increased PsA susceptibility in the South African Indian population. A major limitation of the study is the small sample size in the case-control cohorts, with a low overall statistical power (post-hoc power analysis = 19%). The influence of rs2910164 on miR-146a expression and its role in the pathogenesis of PsA necessitates investigation in a bigger cohort.
2. Remove Table 4 and any text referring to the analysis based on the stratification of patients using clinical parameters, as suggested by the two reviewers.

Table 4 has been removed.

3. Please change running title to “Association of rs29101164 with PsA in South African and Caucasian population”.

Done (lines 16-17)