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SLCO1B1 gene polymorphisms do not influence plasma rifampicin concentrations in a South Indian population

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SUMMARY

OBJECTIVE: To determine the effect of *SLCO1B1* gene polymorphisms (*rs*11045819, *rs*4149032 and *rs*4149033) on rifampicin (RMP) concentrations in adult tuberculosis (TB) patients from south India.

METHODS: We genotyped adult TB patients for three *SLCO1B1* gene polymorphisms—*rs*11045819, *rs*4149032 and *rs*4149033—and compared 2-h post-dosing RMP concentrations of the different genotypes for each of the polymorphisms. Plasma RMP was determined using high-performance liquid chromatography. Genotyping was performed using direct sequencing.

RESULTS: Among the 256 study patients, minor allele frequencies were respectively 0.01 (A), 0.46 (C) and 0.07 (A) for *rs*11045819, *rs*4149032 and *rs*4149033 polymorphisms; genotype distributions followed Hardy-Weinberg equilibrium. RMP concentrations did not significantly differ between the different genotypes of the three polymorphisms.

CONCLUSION: This is the first study to show that *rs*11045819, *rs*4149032 and *rs*4149033 polymorphisms in the *SLCO1B1* gene did not influence RMP concentrations in Indian patients.

KEY WORDS: *SLCO1B1* gene polymorphisms; rifampicin; south India; tuberculosis

RIFAMPICIN (RMP), along with isoniazid (INH), is the mainstay of chemotherapy for the treatment of tuberculosis (TB). RMP displays concentration-dependent activity against *Mycobacterium tuberculosis* in both in vitro and in vivo models.[1–3] The development of RMP resistance and its bactericidal effects are related to peak RMP concentrations.[4,5] Several factors can impact plasma RMP concentrations. Hepatocellular uptake of RMP is mediated by an organic anion-transporter polypeptide 1B1 (OAT1B1) that is coded by the *SLCO1B1* gene.[6] Polymorphisms (*rs*11045819 and *rs*4149032) in this gene have been shown to significantly influence RMP pharmacokinetics and are associated with low RMP exposure.[7–9]

The main aim of the present study was to genotype TB patients for *SLCO1B1 rs*4149032 gene polymorphism and to compare RMP concentrations between the different genotypes of this polymorphism. Similar comparisons in RMP concentrations were made with respect to two other *SLCO1B1* polymorphisms, *rs*11045819 and *rs*4149033.

METHODS

Patients

The study population comprised adult TB patients with pulmonary/extra-pulmonary TB, receiving an RMP-based anti-tuberculosis treatment regimen, who were participating in a large pharmacokinetic study. Those patients whose DNA samples were available took part in this pharmacogenetic study. All patients received thrice-weekly Category I or Category II anti-tuberculosis treatment under the Revised National TB Control Programme (RNTCP) in Chennai Corporation, India. Category I treatment comprised RMP, INH, pyrazinamide (PZA) and ethambutol (EMB) thrice weekly for 2 months, followed by RMP and INH thrice weekly for 4 months. Category II treatment consisted of thrice-weekly RMP, INH, PZA, EMB and streptomycin (SM) for 2 months, thrice-weekly RMP, INH, PZA and EMB for 1 month and thrice-weekly RMP, INH and EMB for 5 months. At the time of study, all patients had received a minimum of 2 weeks of anti-tuberculosis treatment, which was completely supervised.

The study purpose and procedures were explained to the patients, and only those who expressed willingness to participate and provided informed written consent were recruited. The study commenced after obtaining approval from the Ethics Committee of the National Institute for Research in Tuberculosis.

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Table 1 Primers used for genotyping of *SLCO1B1* gene polymorphisms

Polymorphism	Primer sequence (5′→3′)	Remarks
*rs*4149032 and *rs*4149033	TAATCAACTGAAGACTTAGCCCCA	Forward
	ACAGGAACAAGGGCAGTAAAAAC	Reverse
*rs*11045819	TCAACATCGACCTTATCCACTTGT	Forward
	AAACCTGTGTTGTTAATGGGCGAACTGT	Reverse

On the day of the study, anti-tuberculosis drugs were administered under direct supervision and blood was collected at 2-h post-dosing. The blood sample was distributed to vacutainer tubes containing ethylenediaminetetraäcetic acid and heparin; the first was used for DNA extraction and genotyping and the latter for RMP estimation using high-performance liquid chromatography (HPLC).

Genotyping of SLCO1B1 *polymorphisms*

DNA was extracted from whole blood and used for genotyping of three *SLCO1B1* gene polymorphisms: *rs*4149032, *rs*4149033 and *rs*11045819. Genomic DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) and quantitated on Thermo Fisher's NanoDrop2000 spectrophotometer (NanoDrop Technologies Inc, Wilmington, DE, USA). Primers for the three single nucleotide polymorphisms (SNPs) were designed in-house using the National Center for Biotechnology Information's primer designing tool, Primer3 (Table 1). The target regions of interest were amplified on a 9700 GeneAmp™ PCR instrument (Applied Biosystems, Foster City, CA, USA), and the products were sequenced bi-directionally using the Big Dye™ Terminator v. 3.1 sequencing kit (Applied Biosystems) and analysed with a 3100 Avant® automated genetic analyser (Applied Biosystems). Base calling was performed using the SeqScape® v. 2.5 software (Applied Biosystems). Linkage disequilibrium (LD) plots for the three SNPs of the *SLCO1B1* gene were generated using HaploView software v. 4.2 (Broad Institute, Cambridge, MA, USA).

Plasma rifampicin estimation

Blood collected in the heparin vacutainer tube was centrifuged immediately and plasma was separated. Ascorbic acid solution was added to plasma to prevent RMP oxidation. The plasma samples were stored at –20°C until analysis. Plasma RMP concentrations were determined using HPLC according to a validated method.[10] Between- and within-run variations were <10%. The accuracy and precision of the method were respectively 102% and 95%.

Statistical evaluation

Data were analysed using IBM SPSS Statistics, version 20.0 (Statistical Package for the Social Sciences, IBM Corp, Armonk, NY, USA). The Shapiro-Wilks test showed that the pharmocokinetic data were not normally distributed. Values were expressed as median and interquartile range. Non-parametric Kruskal-Wallis test with Bonferroni correction was used to compare multiple groups. $P \leqslant 0.05$ was considered statistically significant.

RESULTS

The demographic characteristics of the 256 TB patients studied are shown in Table 2. Of these, respectively 188, 188 and 255 patients were genotyped for *rs*4149032, *rs*4149033 and *rs*11045819 polymorphisms. The genotype distribution for all the polymorphisms followed Hardy-Weinberg equilibrium; the minor allele frequencies were respectively 0.46 (C), 0.07 (A) and 0.01 (A) for *rs*4149032, *rs*4149033 and *rs*11045819 polymorphisms (Table 3). LD plots showed that the SNPs analysed had a very low linkage disequilibrium with each other in the study population (Figure 1).

The number of CC, CT and TT genotypes of the *rs*4149032 polymorphism were respectively 44, 85 and 59. The median RMP concentrations were respectively 2.93, 2.74 and 2.23 μg/ml in the CC, CT and TT genotypes (Figure 2A). None of the differences in RMP concentrations between the different genotypes was statistically significant. The number of AA, AG and GG genotypes of the *rs*4149033 polymorphism were respectively 1, 25 and 162. The median RMP concentrations were respectively 0.25, 3.54 and 2.75 μg/ml in the AA, AG and GG genotypes (Figure 2B). With respect to the *rs*11045819 gene polymorphism, respectively 4 and 251 patients belonged to the CA and CC genotypes, but none to the AA genotype. The RMP concentra-

Table 2 Patient details

Variables	*n* (%)
Age, years, median [IQR]	39.5 [25.0–50.0]
Body weight, kg, median [IQR]	48.0 [42.0–54.0]
Sex	
Male	165 (64)
Female	91 (36)
Type of TB	
Pulmonary	163 (64)
Extra-pulmonary	93 (36)
Type of anti-tuberculosis treatment	
Category I	210 (82)
Category II	46 (18)

IQR = interquartile range; TB = tuberculosis.

Table 3 Genotype distribution of *SLCO1B1* gene SNPs

	Genotype, *n* (%)			HWE		
rs number	TT	CT	CC	MAF	χ^2	*P* value
*rs*4149032	59 (31.4) GG	85 (45.2) AG	44 (23.4) AA	0.46	1.521	0.217
*rs*4149033	162 (86.2) CC	25 (13.3) CA	1 (0.53) AA	0.07	0.001	0.973
*rs*11045819	251 (98.4)	4 (1.6)	0	0.01	0.016	0.899

SNP = single nucleotide polymorphism; HWE = Hardy-Weinberg equilibrium; MAF = minor allele frequency.

tions in the CA and CC genotypes were respectively 2.43 and 2.93 μg/ml (Figure 2C). Comparison of RMP concentrations among the different genotypes of the *rs*4149033 and *rs*11045819 gene polymorphisms were not carried out, as the test of significance lacked adequate power.

RMP concentrations among the different genotypes of the three polymorphisms were also tested for significance by controlling the third variable (sex, age, body weight); none of the differences in RMP concentrations between the different genotypes was statistically significant.

DISCUSSION

This is the first study from India to examine the influence of three *SLCO1B1* gene polymorphisms on RMP concentrations in TB patients. Weiner et al.'s study in a mixed group of 72 adult TB patients from Africa, North America and Spain reported that the gene polymorphism in *rs*11045819 (463C>A) was associated with lower RMP exposure, and that this polymorphism was more frequent in Black subjects.[7] A South African study in 57 patients reported that those who were heterozygous and homozygous to the *SLCO1B1 rs*4149032 polymorphism had reduced RMP bioavailability.[8] Similar findings have been reported in a study conducted in 57 human immunodeficiency virus (HIV) infected TB patients in Durban, South Africa.[9] In contrast to these findings, a recent study from Tanzania and another from China did not observe any effect of the *SLCO1B1 rs*4149032 genotype on RMP exposure.[11,12] Our findings are in line with these studies, suggesting that studies in different populations are required to further elucidate the effect of *SLCO1B1* genotypes on RMP pharmacokinetics.

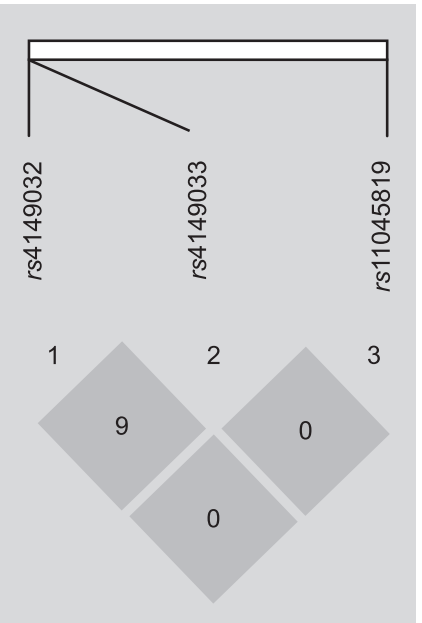

Figure 1 Pairwise linkage disequilibrium between *SLCO1B1* gene SNPs in a South Indian population. The colour coding represents the D'/LOD values; values in cells = r^2 x 100. SNP = single nucleotide polymorphism.

It may not always be possible to collect multiple blood samples in the clinical/field setting for logistical and financial reasons; studies are therefore typically limited to one or two time points. When only one sample can be obtained, the 2-h post-dose RMP concentrations are usually most informative.[13] We therefore estimated drug concentrations at 2-h post-dosing in this study. We undertook a complete pharmacokinetic study in a subgroup of 101 patients and observed that 83.2% of the patients attained peak concentration at 2 h. We further observed a significant correlation between 2-h RMP concentration and peak concentration ($r = 0.8$, $P < 0.001$) and exposure ($r = 0.81$; $P < 0.001$) of RMP (unpublished).

Variant allele frequencies are reported to vary widely across populations. According to a recent review on genetic polymorphisms, the frequency of the A allele in *SLCO1B1 rs*11045819 among Africans, Asians, Caucasians and North Indians was respectively 6.2%, 1.1%, 15.0% and 2.6%.[14] A study from Lucknow, North India, reported an A allele frequency of 4.6%.[15] The present study in a south Indian population has shown this frequency to be 1%. The *SLCO1B1 rs*4149032 polymorphism has been reported to occur in 75% of Nigerians, 70% of South Africans, 29% of Caucasians and 56% of Asians.[8] The frequency of *SLCO1B1 rs*4149032 was observed to be high (76%) in Black Africans.[9] In our study, this frequency was 54%, which is quite similar to that reported in Asians. Although a high propor-

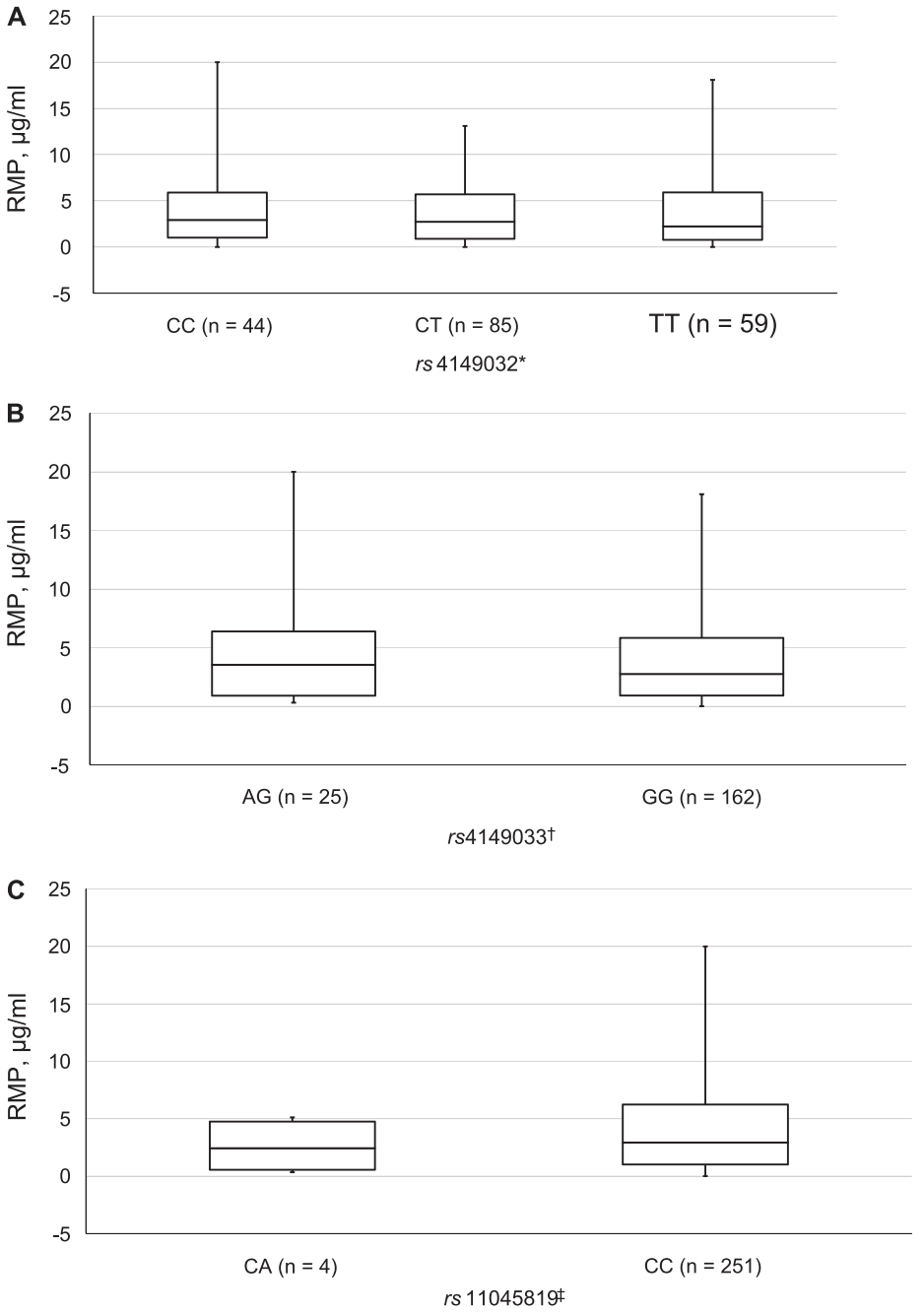

Figure 2 Two-hour RMP concentrations (median [IQR]) in the different genotypes of **A)** *rs*4149032,* **B)** *rs*4149033,† and **C)** *rs*11045819.‡ The edges of the box represent the median [IQR] values; the line extends from the minimum to the maximum values. * Using the Kruskal-Wallis test, differences between *rs*4149032 genotypes were not significant ($P = 0.8493$). † Using the Mann-Whitney *U*-test, differences between *rs*4149033 genotypes were not significant ($P = 0.2652$). ‡ Using the Mann-Whitney *U*-test, differences between *rs*11045819 genotypes were not significant ($P = 0.5229$). RMP = rifampicin; IQR = interquartile range.

tion of South Indians harbour the variant allele of *SLCO1B1 rs*4149032, this is not a matter of concern in the South Indian population due to the absence of any influence of this polymorphism on the pharmacokinetics of RMP. This is the first study to genotype TB patients for another *SLCO1B1* polymorphism, *rs*4149033; we observed that the frequency of the variant A allele was 7% and that the different genotypes did not exhibit any significant difference on RMP concentrations.

A study limitation was that a formal sample size calculation was not done, as we were studying these polymorphisms for the first time in an Indian population. This was a substudy of a large pharmacokinetic study, in which DNA was available for 256 patients. Furthermore, the distribution of the

*rs*11045819 genotypes and *rs*4149033 polymorphisms were highly skewed, and post-power analysis showed less power. However, the *rs*4149032 polymorphism had sufficient power (~80%). As these gene polymorphisms are being studied for the first time in the pharmacogenetic context in the Indian population, further studies are needed to examine this issue in different settings.

In conclusion, plasma RMP concentrations did not differ significantly between the various genotypes of the *rs*4149032 polymorphism of the *SLCO1B1* gene in a South Indian population.

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Conflicts of interest: none declared.

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RESUME

OBJECTIF : Déterminer l'effet des polymorphismes du gène *SLCO1B1* (*rs*11045819, *rs*4149032, et *rs*4149033) sur la concentration de rifampicine (RMP) chez des patients tuberculeux (TB) adultes du sud de l'Inde.

MÉTHODE : Nous avons génotypé des patients TB adultes pour trois polymorphismes des gènes *SLCO1B1*, c'est-à-dire, *rs*11045819, *rs*4149032 et *rs*4149033, et comparé la concentration de RMP 2 h après l'administration en fonction des différents génotypes pour chacun des polymorphismes. La RMP plasmatique a été déterminée par chromatographie en phase liquide à haute performance. Le génotypage a été réalisé par séquençage direct.

RÉSULTATS : Parmi les 256 patients de cette étude, les fréquences des allèles mineurs ont été de 0,01 (A) ; 0,46 (C) ; et 0,07 (A), respectivement, pour les polymorphismes *rs*11045819, *rs*4149032 et *rs*4149033 ; les distributions des génotypes ont suivi l'équilibre de Hardy-Weinberg. Les concentrations de RMP n'ont pas différé significativement en fonction des différents génotypes des trois polymorphismes.

CONCLUSION : Cette étude a montré pour la première fois chez des patients indiens que les polymorphismes *rs*11045819, *rs*4149032 et *rs*4149033 du gène *SLCO1B1* n'influençaient pas les concentrations de RMP.

RESUMEN

OBJETIVO: Determinar el papel de los polimorfismos genéticos del gen *SLCO1B1*, *rs*11045819, *rs*4149032 y *rs*4149033, en las concentraciones de rifampicina (RMP) de los pacientes adultos con diagnóstico de tuberculosis (TB), en el sur de la India.

MÉTODOS: Se llevó a cabo la genotipificación de tres polimorfismos del gen *SLCO1B1*, a saber *rs*11045819, *rs*4149032 y *rs*4149033, en los pacientes adultos con diagnóstico de TB y se compararon las concentraciones de RMP 2 h después de la administración del medicamento en pacientes con los diferentes genotipos, para cada uno de los polimorfismos. La concentración plasmática de RMP se obtuvo mediante cromatografía de líquidos de alto rendimiento. La genotipificación se llevó a cabo por secuenciación directa.

RESULTADOS: En los 256 pacientes que participaron en el estudio, la frecuencia del alelo menos común fue: 0,01 (A); 0,46 (C); y 0,07 (A), para los polimorfismos *rs*11045819, *rs*4149032 y *rs*4149033, respectivamente; la distribución de los genotipos es compatible con el equilibrio de Hardy-Weinberg. Las concentraciones de RMP no exhibieron diferencias estadísticamente significativas en los pacientes con diferentes genotipos de los tres polimorfismos.

CONCLUSIÓN: El presente estudio es el primero en su género que se realiza en pacientes de la India y reveló que los polimorfismos *rs*11045819, *rs*4149032 y *rs*4149033 del gen *SLCO1B1* no influyen en las concentraciones plasmáticas de RMP.