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ASSOCIATION OF XRCC1 GENE POLYMORPHISM AND THE RISK OF LUNG CANCER IN NORTH-INDIAN SUBCONTINENT KASHMIR VALLEY: A POPULATION BASED CASE –CONTROL STUDY.

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ABSTRACT

Background: The x-ray cross-complementing group 1 (*XRCC1*) is mainly involved in base excision repair (BER) of DNA repair pathways. Polymorphism of DNA repair gene *XRCC1* has been identified and it is possible that this polymorphism may affect DNA repair capacity and thus modulate cancer susceptibility. We investigated the relationship between the codon 194 polymorphism in *XRCC1* gene and lung cancer risk in male smokers. **Method**: A population based case-control study of 130 lung cancer patients and 130 healthy control subjects (Individually matched on age and gender) in a Kashmiri population was conducted in Tertiary care super specialty Hospital of Kashmir valley Sheri-Kashmir Institute of Medical Science (SKIMS). *XRCC1* (codon 194) genotype was identified using Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. **Result:** We observed a significantly higher risk of lung cancer among cases who were carriers of the variant genotype Trp/Trp (homozygous mutant) [O.R = 2.43, 95%CI = 1.32 - 4.47, P = 0.006] as compared with homozygous wild genotype Arg/Arg. Our result suggested that the risk for the disease increases significantly as the number of Trp allele increased [OR = 2.31, 95% CI = 1.47 – 3.64, P = 0.0003]. **Conclusion:** Our study revealed that cases, especially smokers with homozygous variant genotype Trp/Trp tend to be more fragile and susceptible to lung cancer [OR = 2.85, 95% CI = 1.27 – 6.41, P = 0.01], as compared to non-smokers [OR = 2.0, 95% CI = 0.73 – 5.43, P = 0.26] and hence may help in identifying individuals at risk in Kashmiri population.

KEYWORDS: *XRCC1*, Polymorphism, Lung cancer, PCR-RFLP, Kashmir, India etc.

INTRODUCTION

Lung cancer is one of the most common malignant neoplasm worldwide, accounting for more deaths than any other cancer. Lung cancer is broadly classified into two groups: non-small cell lung carcinoma (NSCLC) and small-cell lung carcinoma (SCLC) based on histopathological factors. Their incidence is increasing globally at a rate of 0.5% per year.[1]

Lung cancer remains the most lethal form of cancer in men. In U.S, lung cancer has now surpassed breast cancer as the most common cause of cancer-related deaths in women. Lung cancer constituted 14.4% of all cancers, according to a review of 9210 consecutive autopsies reported by Banker in 1957.[2] Lung cancer was reported to be the second most common malignancy in an earlier hospital based study from Kashmir valley of the Indian subcontinent. Non-small cell lung carcinoma accounts for 85% and small-cell lung carcinoma accounts for 15% to 20% of cases.[3]

Lung cancer results from complex interactions between many genetic and environmental factors.[2] Environmental factors such as tobacco smoke, dietary factors, infectious agents and radiation add to the carcinogenic load to which the humans are exposed. Cigarette or hookah smoking accounts for an estimated 30% of all lung cancer cases and >85% of deaths from lung cancer.[4,5,6] Cigarette smoke contains over 4000 chemical compounds such as carcinogens and genotoxicants including oxidants which inflicts oxidative DNA damages.

A critical cellular response that counteracts the carcinogenic effects of DNA damages is DNA repair. DNA repair systems are fundamental to the maintenance of genomic integrity in the face of replication errors, environmental insults and the cumulative effects of age. Individuals with DNA repair defects might be at higher risk of cancer.[7,8] In humans >70 genes are involved in the five major DNA repair pathways: direct repair, BER,

NER, mismatch repair and double strand break repair.[9, 10] The DNA repair protein X-ray repair cross-complementing group 1 (XRCC1) acts as a facilitator or coordinator in BER of oxidative DNA and single-strand break repair (SSBR) in mammalian cells and forms a repair.[11] The human XRCC1 gene, located on chromosome 19q13.2, encodes for a 633 amino acids protein. XRCC1 protein interacts with many components of BER such as DNA polymerase ß, APE1, hoGG1, poly (ADP-Ribose) polymerase and DNA ligase III in the NH_2-terminal, central and COOH-terminal regions respectively.[12-14] A lot of information about XRCC1 function has been derived from mutant mammalian cell lines. XRCC1 mutants were initially identified in the AA8 strain of Chinese hamster ovary (CHO) cells and four of these denoted EM7, EM9, EM-C11 and EM-C12, represent a model to study the consequences of the lack or a reduced level of this protein.[15] In 1998 Shen *et al.*,[16] described three polymorphisms of XRCC1 gene, which resulted in non-conservative amino acid changes at evolutionary conserved regions: C →T substitution in codon 194 of exon 6 (Arg to Trp); G→A substitution in codon 280 of exon 9 (Arg to His) and G→A substitution in codon 399 of exon 10 (Arg to Gln).

Earlier studies have reported the relationship of XRCC1 polymorphism at codon 194 and codon 399 and cancer risk or carcinogen-DNA adducts.[17,18] Recently polymorphism in relation to lung cancer risk in Korean, African, Americans and Caucasians were observed.[19, 20] Lunn et *al*[21] has shown that the frequency distribution of these two polymorphisms of XRCC1 varied remarkably in Caucasians and in Taiwanese. In Kashmir, lung cancer occurs predominantly in male smokers and squamous cell lung carcinoma is the most frequent histological type, which may be due to a very high smoking rate among males (19.34 per 100 000).[22] During our research work, we didn't happen to find any female patient suffering from lung cancer risk as the number of women having such disease in Kashmir is remarkable less which may be due to very less smoking rate among females[22] and also the epidemiological characteristics of this valley is notably different from those of western countries.

DNA repair is well known as a ‘‘double-edged sword’’ in cancer studies. Epidemiological evidence supports that DNA repair capacity is one of the determinants of genetic susceptibility to Cancer.[48-50] This study implicates that BER including *XRCC1*, may be the major pathway for removing the mutagenic DNA damages arising from procarcinogens in cigarette or hookah smoke. Although it is difficult to attribute the carcinogenicity of tobacco to any particular compound, most important causative agents for squamous cell carcinoma are PAHs, such as benzo(a) pyrene.[51,52] A variety of reactive oxygen species, such hydroxyl radical and hydrogen peroxide are generated during enzymatic oxidation of PAHs.[53,54] Oxidative DNA damages are primarily removed via BER, including XRCC1. In addition to this, BER also targets depurinating DNA adducts, such as N7-methylguanine and N3-methyladenine, derived from radical cations formed by one-electron oxidation of PAHs.[55] Another reason for the association between squamous cell carcinomas and XRCC1 polymorphism may be that interaction with other pro-carcinogens induced DNA damage (56,57). This is most likely to happen for exposure to cigarette smoke, where there are many potent pro-carcinogens producing various DNA damages. It is important to integrate DNA repair process with DNA damage checkpoints and cell survival, to evaluate the role of DNA at both cellular and organismic levels. Hence, there might be a protective role of *XRCC1* polymorphisms in cancer due to enhanced efficiency of apoptosis at a cellular level as a result of diminished DNA repair capacity secondary to the genetic polymorphisms.[31, 32, and 58]

To determine whether the *XRCC1* 194Trp allele is a risk factor for lung cancer in Kashmir, we performed a case-control study to examine this hypothesis.

MATERIALS AND METHODS

Study subjects

In this case-control study, the case group consisted of 130 diagnosed patients with histologically confirmed lung carcinoma from the department of cardiovascular and thoracic surgery in a Sheri Kashmir institute of medical sciences (SKIMS) Srinagar. Patients given chemotherapy treatment were excluded. The control group comprised of 130 healthy volunteers having no previous history of lung cancer or any other cancer type elsewhere in the body. They were obtained from community centers and other departments of SKIMS and were individually matched to the cases by age, gender and smoking status (age ± 10 years). Data on age, gender, smoking status and amount were derived from questionnaires (table 1). To be considered a smoker, individuals must have smoked at least once a day for > 1 year in his lifetime and those who have never smoked were taken as non-smokers. At recruitment, informed consent was obtained from each subject. The collection of blood samples for this study was approved by the appropriate institutional Ethics Committees.

Genotyping

Genomic DNA was extracted from blood samples using modified salting-out method.[23] *XRCC1* genotype was determined by a PCR-RFLP assay. PCR primers [GenBank accession no. L30479] were 5'-GCCCCGTCCCAGGTAAG-3' [bases 27775-27794 of XRCC1] and 5'-AGCCCCAAGACCCTTTCACT-3' [bases 28370 - 27794 of XRCC1], which generate a 494-bp fragment. PCR was performed in a total volume of 25µl carried out in 0.2ml PCR tubes (axygen). The PCR reaction mixture consisted of 50-100ng of genomic DNA templates. 200µM of deoxynucleotidetriphosphate [dNTPs] (Biotools), 0.5µM of each primer (Fermentas), 2.5mM $MgCl_2$ and 2.0U of Taq Polymerase with 2.5µl

10x reaction buffer (Biotools). For amplification, PCR programs initiated by a 5 min denaturation step at 94°C followed by 35 cycles of 30s at 94°C which is followed by annealing step of 20s at 60°C, 30s for extension step at 72°C and a final elongation step of 72°C for 10 min. PCR product (494-bp) was then resolved on 1.5%agarose gel (Sisco Research Lab Pvt. Ltd). The PCR products (494 bp) were digested overnight with 10 units of MspI (Fermentas) at 37oC. The digestion product was then resolved on 2% agarose gel (Sisco Research Lab Pvt Ltd) containing ethidium bromide and then evaluated using a gel doc system (AlphaimagerTM 2200, Alpha Innotech Corporation).

The enzyme MspI recognizes the wild allele of codon 194. It has two recognition sites on the 494-bp fragment at the positions 174 and 198 of which the position 198 is the polymorphic site. Thus the wild type genotype of codon 194 Arg/Arg generates three fragments 292, 174 and 24 bp, while the homozygous mutant genotype Trp/Trp lead to two fragments of 313 and 174 bp and the heterozygous variant genotype Arg/Trp resulted in the formation of three bands of 313, 292 and 174 bp (Fig. 1). The 174 bp fragment from the digestion of the 494 bp fragment is always present irrespective of the genotype and was used as an internal control for complete digestion.

Statistical Analysis

The allelic frequencies were estimated by gene counting and genotypes were scored. The chi-square (χ^2) test was used to examine differences in demographic variables, distribution of genotypes with those expected for a population in the Hardy-Weinberg equilibrium and to test the significance of the differences of observed alleles and genotypes between groups. The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by using a yates' continuity corrected chi-square (χ^2) test and based on this calculation, risk of lung cancer was estimated. Statistical analysis of the data was performed using Graph Pad Prism version 5.0 software. The criterion for statistical significance was defined as $P < 0.05$.

RESULTS

In this work, we investigated a common single nucleotide polymorphism of *XRCC1* gene Arg 194 Trp and its association with lung cancer. The genotypic analysis of this single nucleotide polymorphism of the *XRCC1* gene for 130 lung cancer cases and 130 healthy subjects (controls) in Kashmiri population was performed using PCR - RFLP method. The characteristic data of cases and controls according to age, gender, smoking status and histological type are shown in table 1.

Table 1: General characteristics of the population.

Variables	Cases (n = 130) No. (%)	Controls (n = 130) No. (%)
Age (yrs) (mean ± SD)[a]	57 ± 10	58 ± 10
< 60	60 (46.1)	77 (59.2)
> 60	70 (53.8)	53 (40.7)
Gender		
Male	130 (100.0)	130 (100.0)
Smoking status		
Ever	90 (69.2)	75 (57.6)
Never	40 (30.7)	55 (42.3)
Histological type		
Squamous cell ca[b]	58 (44.6)	
Adenocarcinoma	27 (20.7	
Others[c]	45 (34.6)	

([a]Median of age in study subjects, [b]Carcinoma, [c]other includes non-differentiated cancer, bronchioalveolar carcinoma and mixed cell carcinoma).

The mean age was similar between cases (57±10 years, range 25-70) and controls (58±10 years, range 25-70) in the study. The distribution of genotypes and alleles of XRCC1 among cases and controls and its association with lung cancer risk is summarized in table 2-5. The distribution of genotypes was in Hardy-Weinberg equilibrium. When the cases were categorized by histological type, the frequencies of Arg/Arg, Arg/Trp and Trp/Trp genotype in the squamous cell carcinoma group (36.2, 13.7 and 50.0%, respectively) were significantly different from those among controls (59.7, 22.2 and 13.8%, respectively, *P < 0.05*). The frequencies of genotypes in the adenocarcinoma group and the one labeled as the others group were not statistically significant as compared with controls.

Table 2: *XRCC1* genotype and allelic frequency among cases.

	Genotype			
	Arg/Arg	**Arg/Trp**	**Trp/Trp**	**Trp allele frequency (%)**
Control	72 (59.7)	35 (22.2)	23 (13.8)	26.1
Cases	58(44.6)	27 (20.7)	45 (34.6)	45.0
SQ[a]	21 (36.2)	8 (13.7)	29 (50.0)	56.8
AD[b]	14 (51.8)	5 (18.5)	8(29.6)	38.8
Others[c]	21 (46.6)	15 (33.3)	9 (20.0)	36.6

[a] Squamous cell carcinoma; *OR* = 4.32, *CI* = 2.08 to 8.99, *P* = 0.0001, control *versus* squamous cell carcinoma, [b]Adenocarcinoma; *OR* = 1.79, *CI* = 0.66 to 4.80, *P* = 0.37, [c]Non-differentiated cancer, bronchio-alveolar carcinoma and mixed cell carcinoma; *OR* = 1.34, *CI* = 0.54 to 3.33, *P* = 0.70.

Table 3: Genotypic and allelic frequencies of *XRCC1* codon 194 in cases and controls and relative risk of lung cancer (n = 130).

Variants	Case (%)	Control (%)	O.R[a](95% CI)	[b]χ^2	P value
Arg/Arg	58 (44.6)	72 (59.7)	1	-	1
Arg/Trp	27 (20.7)	35 (22.2)	0.95 (0.52 - 1.76)	0.019	0.98
Trp/Trp	45 (34.6)	23(13.8)	2.43 (1.32 - 4.47)	7.47	0.0063
Arg	143 (55.0)	102 (73.9)	1	-	1
Trp	117 (45.0)	36 (26.1)	2.31(1.47 - 3.64)	12.84	0.0003
Arg/Trp + Trp/Trp	72(55.3)	58 (44.6)	1.54 (0.94 - 2.51)	2.6	0.10

[a]O.R calculated using GraphPad Prism 5.0.
[b] $\chi 2$ value calculated using Pearson's chi-square test.

Table 4. Association between XRCC1 codon 194 genotypes and lung cancer, according to age in years.

≤ 60				≥ 60			
Case (%)	Control (%)	O.R (CI)	P Value	Case (%)	Control (%)	O.R (CI)	P
33 (56.6)	45 (71.2)	1	-	25 (35.7)	27 (50.9)	1	-
13 (21.6)	17 (22.1)	1.04 (0.44 - 2.44)	0.90	14 (20.0)	18 (33.9)	0.84 (0.34 - 2.03)	0.87
14 (23.3)	15 (19.4)	1.27 (0.54 - 2.99)	0.78	31 (44.2)	8 (15.1)	4.18 (1.62 - 10.8)	0.004
27 (45.0)	32 (41.5)	1.15 (0.58 - 2.27)	0.81	45 (64.2)	26 (49.1)	1.86 (0.90 - 3.86)	0.13

Table 5: Association between *XRCC1* codon 194 genotypes and lung cancer, according to smoking status.

Genotype	Ever[a]				Never			
	Case (%)	Control (%)	O.R (CI)	P Value	Case (%)	Control (%)	O.R (CI)	P
Arg/Arg	42 (46.6)	40 (53.3)	1	-	16 (40.0)	32 (58.2)	1	
Arg/Trp	15 (16.6)	24 (32.0)	0.59 (0.27 - 1.29)	0.26	12 (30.0)	11 (20.0)	2.18 (0.79 - 6.02)	0.20
Trp/Trp	33 (36.6)	11 (14.6)	2.85 (1.27 - 6.41)	0.01	12 (30.0)	12 (21.8)	2.00 (0.73 - 5.43)	0.26
Arg/Trp + Trp/Trp	48 (53.3)	35 (46.6)	1.30 (0.70 - 2.41)	0.48	24 (40.0)	23 (41.8)	2.08 (0.91 - 4.78)	0.12

[a]Subjects who smoke at least once a day were considered as smokers

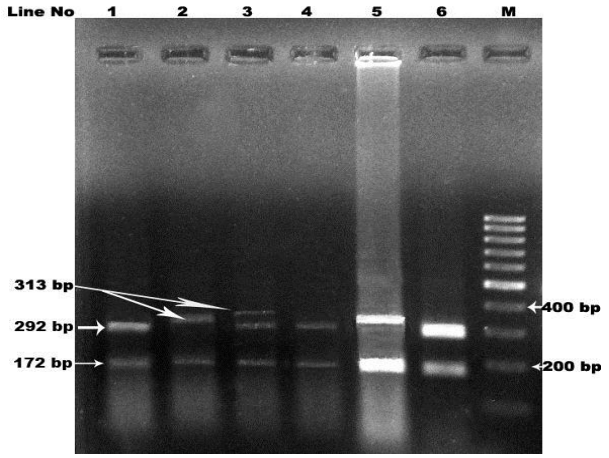

Fig 1: RFLP analysis of the *XRCC1* Arg194Trp polymorphism.

Lane no.1, 4 and 6 shows Arg/Arg wild genotype, lane no. 2 and 5 denotes Trp/Trp homozygous mutants and lane no.3 represent Arg/Trp heterozygous variant respectively. Lane M is the DNA size marker.

Table 3 shows the ORs and 95% CI for all lung cancer cases and controls by XRCC1 genotypes and alleles. When the Arg/Arg genotype was used as the reference group, the Trp/Trp genotype was associated with elevated as well as statistically significant risk for all lung cancer [OR = 2.43, CI: 1.32 - 4.47, P = 0.0063]. The frequency of Arg/Trp genotype was similar to those of controls. When analyses were stratified by histological type, the presence of at least one Trp allele was associated with a significant increased risk for squamous cell carcinoma [OR = 4.32, CI = 2.08 to 8.99, P = 0.0001]. The overall risk for lung cancer disease increased as the number of Trp alleles increased [Trp allele = 2.31, CI: 1.47 - 3.64, P = 0.0003]. The association between *XRCC1* genotype and lung carcinoma, according to smoking status is shown in table 5. In our study, we observed that frequencies of

genotypes Arg/Arg, Arg/Trp and Trp/Trp in ever smokers (46.6, 16.6 and 36.6%, respectively) were different from those among controls (53.3, 32.0 and 14.6%, respectively) with Trp/Trp genotype being significantly associated with borderline increased risk for lung cancer disease [OR = 2.85, CI: 1.27 - 6.41, P = 0.01] especially squamous cell carcinoma as shown in table 3. In the group of individuals who have never smoked in their life span, the distribution of XRCC1 genotypes was not significantly different between lung cancer cases and controls.

When analyses was stratified by age as shown in table 4, no significant deviation was observed for the distribution of *XRCC1* genotypes and Trp allele frequencies among cases and controls belonging to ≤ 60 years age group, however, in the group of individuals having ≥ 60 years of age, the Trp/Trp genotype was associated with a significantly increased risk for lung cancer as compared with those of controls [OR = 4.18, CI: 1.62 - 10.8, P = 0.004]. This shows that the homozygous variant genotype Trp/Trp of *XRCC1* codon 194 could be considered for further risk assessment.

DISCUSSION

Although some research has been carried out to elucidate the role of *XRCC1* in lung cancer.[28, 29, 30] However, no report regarding the role of this gene in lung cancer is available from this region. In this work, we examined an SNP of XRCC1 gene (Arg194Trp) as a candidate susceptibility gene for lung cancer in a population based case-control study in Kashmir valley for the first time. We found a positive and statistically significant association between XRCC1 codon 194 genotypes and lung cancer, especially Trp/Trp genotype among the Kashmiri population. These findings suggest that the 194 Trp allele could be used as a biomarker for genetic susceptibility to lung cancer in smokers. The polymorphism chosen for this study has been shown to have functional significance and may be responsible for a low DNA repair capacity and phenotypic characteristic of cancer patients including lung carcinoma.[24-27]

A few studies done previously suggested that Arg194Trp polymorphism was associated with a reduced risk of squamous cell carcinoma of the pharynx, oral cavity and other cancer related to tobacco and alcohol consumption.[17,31] The different results in different populations may be because of genetic and environmental differences.[32] The prevailing concept is that defect in one or more steps of DNA repair pathways may be an important determinant in carcinogenesis. Three coding polymorphisms at conserved sites have been reported in the *XRCC1* gene.[19] In our case-control study, we focused on the codon 194 Trp polymorphism and its association with lung cancer risk. Each polymorphism in the 194 codon of the human *XRCC1* gene was composed either of two types of alleles- the wild type (Arg) or the polymorphic variant type (Trp) with different RFLP size distributions. Arg/Arg, Arg/Trp and Trp/Trp genotypes represent wild/normal, heterozygous variant and homozygous variant respectively. We demonstrated that out of these genotypes, the frequency of homozygous variant genotype Trp/Trp was significantly higher for codon 194 of XRCC1 gene [34.6% vs. 13.8%, χ^2 = 7.47, P = 0.0063] in cases as compared to control subjects. Also the Trp allele was found to be associated with an increased risk for squamous cell carcinoma of the lung.

The effect of *XRCC1* gene polymorphism is still not clear and results up to now are inconsistent.[28,32] Studies investigating the association between polymorphisms and lung cancer risk have also led to contradictory results. Our findings are consistent with few studies that reported positive association between Trp 194 carriers and lung cancer risks[33-37] and other cancer like breast cancer risk[38-40], head and neck squamous cell carcinoma.[41,42], esophageal carcinoma[43], colorectal carcinoma[44] etc., whereas most studies observed overall no or an inverse association between Trp194 carriers and lung cancer risk and other types of carcinomas too.[17,18,20,29,30] The different frequencies of codon 194 may account for their different contribution to lung cancer risk. It is biologically plausible to assume that *XRCC1* polymorphism at codon 194 may have functional significance. This polymorphism occurs at conserved evolutionary sites and the mutation results in amino acid substitutions[16] which may alter the structure of DNA repair enzyme and accordingly may be associated with a deficiency in DNA repair capacity. However, the size of our study is a primary limitation and the mechanisms of the *XRCC1* codon 194 polymorphism to lung cancer risk need to be further explored in a larger Kashmiri population.

In this case-control study, we also observed that the cases having ≥ 60 years of age show significantly higher frequency of variant genotype 194 Trp [44.2% vs. 15.1%, P = 0.004]. Moreover, the distribution of patients into various subtypes of lung cancer also indicates that the majority of patients suffered from squamous cell carcinoma (Table 2). Genetic susceptibility to lung cancer may depend on the level of exposure to tobacco smoke.[28, 29] Therefore, we examined further association between tobacco smoke exposure and the distribution of *XRCC1* genotypes. We observed that cases who smoke, have significantly higher frequency of 194 Trp variant [36.66% Vs. 14.66%, P = 0.01] as compared with controls and an increased risk of about two-folds in case of subjects (cases). It is possible that such a finding is attributable to chance because of the relatively small number of sample size.

CONCLUSION

Our study suggests that a codon 194Trp allele of the *XRCC1* gene was associated with an increased risk of lung cancer, especially squamous cell carcinoma of the lungs in smokers, suggesting a possible role for *XRCC1* Arg194Trp polymorphism in identifying individuals at

risk of developing lung cancer. However, the evaluation of the impact of the polymorphism of XRCC1gene on the development and prognosis of the disease has to be explored in the future, concentrating on subpopulations, that may experience greater exposure to various DNA-damaging agents.

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AUTHOR'S CONTRIBUTION

GQM has contributed in concept, design, clinical studies, experimental studies, data acquisition, statistical analysis, drafting, final approval and guarantor. BAG has contributed in concept, literature review, experimental studies, guarantor and final approval, SS has contributed in design, drafting, clinical studies and final approval, AGA has contributed in concept, design, intellectual content, drafting, guarantor and final approval, HB has contributed in concept, design, manuscript review, manuscript preparation, manuscript editing, drafting, guarantor and final approval. AM have contributed in concept, design, clinical studies, drafting, final approval and guarantor.

COMPETING INTERESTS

The authors have declared that no competing interest exists.

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