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ORIGINAL RESEARCH

Genetic Evidence Supporting Causal Roles of mTOR-Dependent Proteins in Rheumatic Fever: A Two-Sample Randomized Mendelian Study

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ABSTRACT

Background: The expression of signaling molecules downstream of the mammalian target of rapamycin (mTOR) is dysregulated in patients with rheumatic fever (RF), but the causality of mTOR on RF remains unknown. This study aimed to investigate the causal effects of the mTOR-dependent proteins in RF.
Methods: The summary data for targets of the mTOR signaling were acquired from the publicly available INTERVAL study GWAS data. Data on RF have been obtained from the Integrated Epidemiology Unit GWAS database (38,209 cases and 156,711 healthy controls). A two-sample Mendelian randomization (MR) study was conducted to examine the association of RF risk and mTOR-dependent proteins (EIF4EBP2, EIF-4E, EIF-4G, EIF-4A, RP-S6K, and ATG7), including the inverse-variance weighted (IVW) method, MR-Egger, and weighted median, which was followed by sensitivity analyses.
Results: RP-S6K is associated with a lowered risk of RF with an odds ratio (OR) of 0.97, 95% confidence interval (95% CI) of 0.94–0.99 ($p = 0.027$). In contrast, ATG7 accounts for higher risk of RF with an OR of 1.05 (95% CI = 1.00–1.12, $p = 0.047$). No apparent heterogeneity and no horizontal pleiotropy were observed in the sensitivity analysis ($p > 0.05$). No statistical significance was identified for levels of EIF4A, EIF4G, EIF4E-BP2, and RP-S6K with RF risk ($p > 0.05$).
Conclusion: MR found robust evidence of a causal association between RF and mTOR.

Yan-Fei Mu and Qian Wang have contributed equally to this work and share the first authorship.

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RP-S6K and ATG7 may be targeted for intervention by repurposing existing therapeutics to reduce the risk of RF.

Keywords: Rheumatic fever; Mammalian target of rapamycin (mTOR); Mendelian randomization; Single nucleotide polymorphism; Risk factor

Key Summary Points

Why carry out this study?

RF is a pathological autoimmune response to throat infection with group A *Streptococcus*, which remain a public health problem in many low-income countries. However, the etiology and pathogenesis of RF are still unclear.

MTOR is known to regulate cell growth, proliferation, and survival. Rapamycin is currently being developed to treat autoimmune diseases.

The causality of mTOR on RF remained unknown, so this study aimed to investigate the causal effect of MTOR-dependent proteins in RF.

What was learned from the study?

By performing Mendelian randomization analysis, we found a causal relationship between a mTOR-related target (RP-S6K and ATG7) and RF risk, which is that higher circulating levels of RP-S6K may be causally associated with lower RF risk, and higher circulating levels of ATG7 may be associated with higher RF risk.These findings may provide new insights into the intervention of RF.

INTRODUCTION

Rheumatic fever (RF) is a pathological autoimmune response to throat infection with group A *Streptococcus* (GAS) [1]. The illness is characterized by various combinations of joint pain and swelling, cardiac valvular regurgitation with the potential for secondary heart failure known as rheumatic heart disease (RHD), chorea, subcutaneous nodules, erythema marginatum, and fever [2, 3]. The mortality rate of RF and its sequel, RHD, is comparable to that of rotavirus, and about 50% of that of malaria [4]. There are approximately 471,000 cases of acute rheumatic fever (ARF) each year, and approximately 350,000 annual deaths as a result of ARF or RHD [2]. It is estimated to cause a USD2.2 trillion loss worldwide annually [5]. Thus, it is imperative to determine causal associations and to develop valid strategies to illustrate the pathogenesis that can help reduce the risk of RF.

The mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase in the phosphoinositide 3-kinase related kinase family that is the core catalytic component of two distinct protein complexes, known as mTOR Complex 1 (mTORC1) and 2 (mTORC2) [6, 7].The mTOR signaling controls various cellular processes, including apoptosis, autophagy, translation, energy metabolism, and inflammation [8]. Therefore, the regulation of mTOR provides a new therapeutic strategy for various diseases. However, the role of mTOR in RF is still not fully clear.

At present, rapamycin has achieved promising progress in alleviating inflammatory autoimmune diseases, including mouse models of lupus and multiple sclerosis [9, 10]. MTORC1 is a central hub of metabolic and inflammatory signaling, which initiates biosynthetic pathways for cell growth and proliferation via phosphorylation of ribosomal S6K and eukaryotic initiation factor 4E (eIF4E). It is also known to suppress autophagy, which is critical to providing energy and molecular building blocks by recycling macromolecules in response to nutrient and environmental stress [11, 12]. However, the causality from mTOR on RF remains unknown. This study mainly explores the causal relationship between mTOR and RF.

Mendelian randomization (MR) is an analytical approach that utilizes genetic variants, generally single nucleotide polymorphisms (SNPs), as instrumental variables for exposure to diminish confounding and reserve causality, thereby strengthening the causal inference of

an exposure-outcome association, which can overcome confounding biases inherent in observational studies. In this study, we investigated whether the risk of RF varied with proteins related to mRNA translation in mTORC1 signaling (EIF4EBP2, EIF-4E, EIF-4G, EIF-4A, and RP-S6K), and proteins associated with Inhibiting protein catabolism in mTORC1 signaling (ATG7).

METHODS

Genetic Datasets

Genetic prediction of the exposure of the mTOR-related GWAS data was obtained from the publicly available INTERVAL study Proteomics GWAS data. The study quantified 3,622 plasma proteins in 3,301 healthy participants from the INTERVAL study in whom a SomaLogic aptamer-based plasma protein assay had been run [13]. https://www.phpc.cam.ac.uk/ceu/proteins/ We applied the genetic predictors of mTOR downstream targets, namely EIF4EBP2, EIF-4E, EIF-4G, EIF-4A, RP-S6, and ATG7, to determine the causal association between mTOR-downstream targets and RF.

The outcome dataset was obtained from the Integrative Epidemiology Unit GWAS database (https://gwas.mrcieu.ac.uk), which includes 38,209 cases and 156,711 healthy controls of European ancestry. The database was developed at the MRC Integrative Epidemiology Unit at the University of Bristol. This resource is a manually curated collection of complete GWAS summary datasets made available as open-source files for download.

Selection of genetic instruments

The exposure data with known genetic determinants are also called instrumental variables (IV) [14]. We applied the genetic predictors of mTOR downstream targets, namely EIF4EBP2, EIF-4E, EIF-4G, EIF-4A, RP-S6K, and ATG7, to determine the causal association between mTOR-downstream targets and RF. Firstly, we chose SNPs that reached a genome-wide significance level ($p < 5 \times 10^{-6}$), identifying the SNPs relevant to EIF4EBP2, EIF-4E, EIF-4G, EIF-4A, RP-S6K and ATG7. To prevent bias of results for strongly associated SNPs and to ensure that SNPs were valid and independent, the linkage disequilibrium threshold for clumping was set to $R^2 < 0.001$. We evaluated the strength of the gene instrument from the F-statistic using an approximate approach, excluding SNPs with F-statistic < 10 to eliminate weak instrument bias.

In addition, Phenoscanner (http://www.phenoscanner.medschl.cam.ac.uk/) analyzed a curated database of publicly available results from large-scale genetic association studies [15]. To see whether these SNPs were associated with potential risk factors, including smoking and drinking, we removed SNPs related to any potential confounders at genome-wide significance (Fig. 1).

Statistical Analyses

We performed the MR analysis using the fixed-effects inverse-variance weighted (IVW) method as the primary analysis to evaluate the causal associations between mTOR-downstream targets and RF. This method provided the most robust causal estimates while being relatively sensitive to pleiotropy. Thus, the weighted median method, the MR-Egger regression, and the MR Pleiotropy Residual Sum and Outlier method were further applied as supplementary analyses [16, 17]. Egger regression, a tool to detect slight study bias in meta-analysis, can be adapted to test for bias from pleiotropy. We further assessed the horizontal polytropic using the MR-Egger intercept test and leave-one-out analyses. Cochran's Q test was also used to identify heterogeneity. In addition, the leave-one-out sensitivity test was carried out, which mainly calculates the MR results of the remaining IVs after removing IVs one by one. If the MR results estimated by other IVs after removing an IV significantly differ from the total results, it indicates that the MR results are sensitive to the IV. All statistical analyses were conducted using R (v.4.2.1), with the MR analysis performed using the 'TwoSampleMR' package.

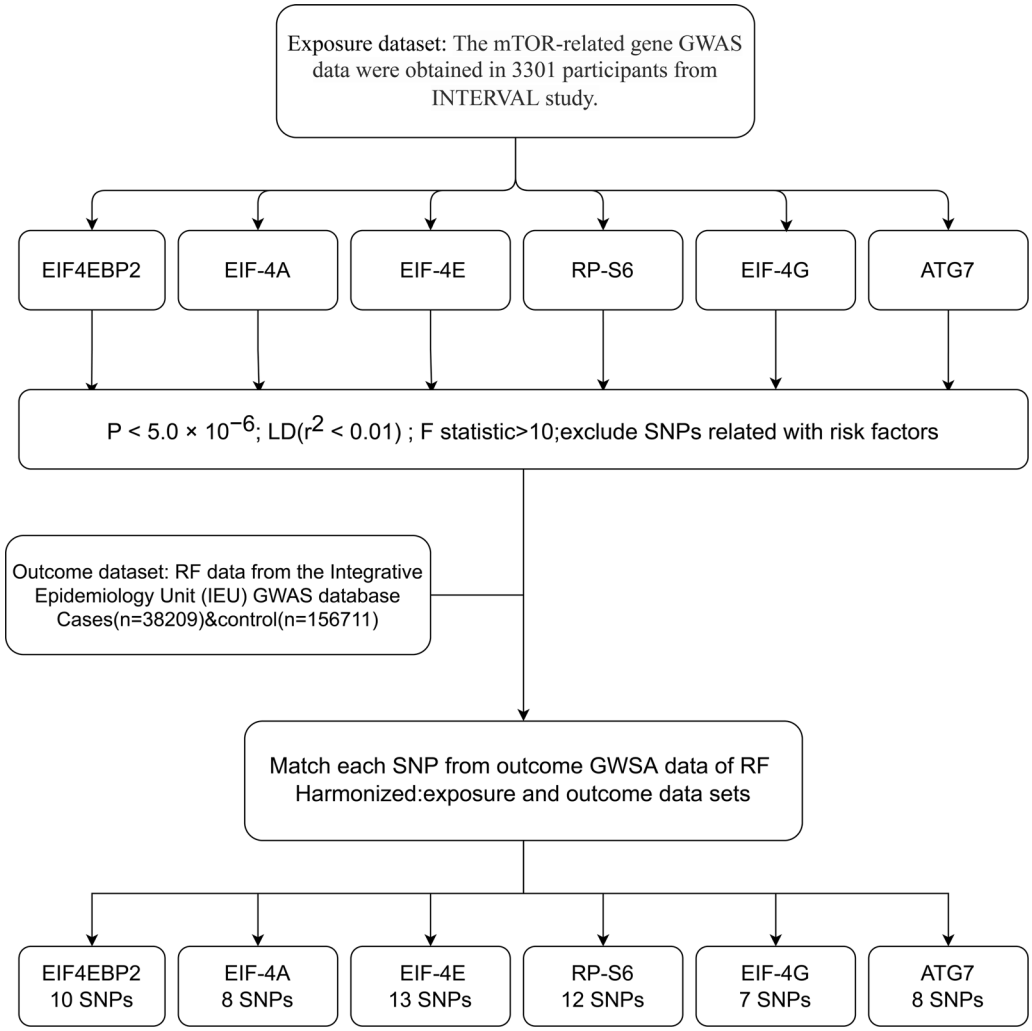

Fig. 1 Flow chart and the data selection process. A series of quality controls were performed on the exposure data, and the exposure and outcome data were coordinated to screen out independent SNPs for subsequent analyses. *RP-S6K* ribosomal protein-S6 kinase, *EIF4E-BP* eukaryotic translation initiation factor 4E-binding protein, *eIF4E* eukaryotic translation initiation factor 4E, *eIF4A* eukaryotic translation initiation factor 4A, *eIF4G* eukaryotic translation initiation factor 4G, *ATG7* autophagy related protein 7

RESULTS

As mentioned earlier, for genetic exposure prediction, we obtained SNPs that strongly and independently predicted EIF4EBP2, EIF-4E, EIF-4G, EIF-4A, RP-S6K, and ATG7 circulating plasma levels from a GWAS of 3,301 participants in the INTERVAL study [13]. After a series of quality control steps, 15, 16, 9, 11, 16, and 10 independent SNPs ($p < 5.0 \times 10^{-6}$, $r^2 < 0.01$) were associated with outcome RF (Supplementary Table 1).

In the IVW analyses, RP-S6K cap-dependent translation factor circulating level was associated with a lower risk of RF with an [odds ratio (OR) = 0.97, 95% confidence interval (CI) = 0.94–0.99, $p = 0.027$]. Meanwhile, the estimates were very similar in a sensitivity analysis. MR analyses based on the methods of MR-Egger analysis (OR = 0.96, 95% CI = 0.90, 1.03; $p = 0.273$), and weighted median (OR = 0.98, 95% CI = 0.95, 1.02; $p = 0.408$). The Cochran Q test-derived p value was 0.344, indicating no obvious heterogeneity. The

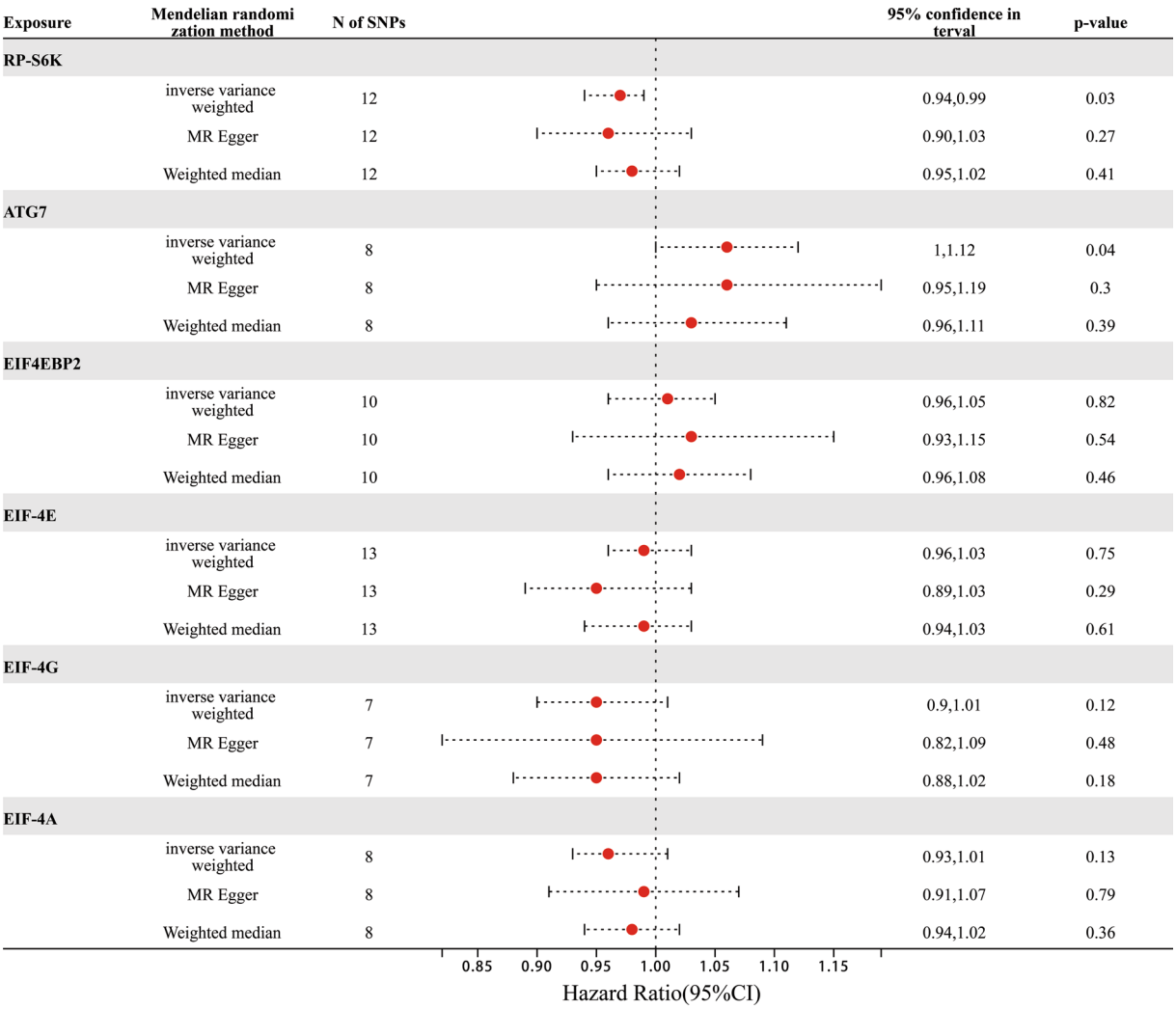

Fig. 2 The causal associations between mTOR and RF were evaluated by the odds ratio (OR) values of IVW, MR Egger, and weighted median. *RP-S6K* ribosomal protein-S6 kinase, *EIF4E-BP* eukaryotic translation initiation factor 4E-binding protein, *eIF4E* eukaryotic translation initiation factor 4E, *eIF4A* eukaryotic translation initiation factor 4A, *eIF4G* eukaryotic translation initiation factor 4G, *ATG7* autophagy related protein 7

pleiotropy test showed no horizontal pleiotropy ($p = 0.907$). The leave-one-out sensitivity analysis suggested that the MR analysis result was not dramatically driven by any single SNP.

ATG7 was associated with a higher risk of RF (OR = 1.05, 95% CI = 1.00, 1.12, $p = 0.047$). Meanwhile, the estimates were very similar in a sensitivity analysis. MR analyses based on the methods of MR-Egger analysis (OR = 1.06, 95% CI = 0.95–1.19; $p = 0.300$), and weighted median (OR = 1.03, 95% CI = 0.96, 1.11; $p = 0.387$). Similarly, heterogeneity ($p = 0.536$) and pleiotropy ($p = 0.857$) were not detected. The leave-one-out sensitivity analysis suggested that the MR analysis result was not dramatically driven by any single SNP.

EIF4EBP2 and EIF-4E were not associated with RFo (OR = 1.01, 95% CI = 0.95, 1.05, $p = 0.828$) and (OR = 0.99, 95% CI = 0.96, 1.03, $p = 0.758$) Similarly, EIF-4G and EIF-4A were not associated with RF (OR = 0.95, 95% CI = 0.90, 1.01, $p = 0.129$) and (OR = 0.97, 95% CI = 0.93, 1.01, $p = 0.136$) (Figs. 2, 3, 4).

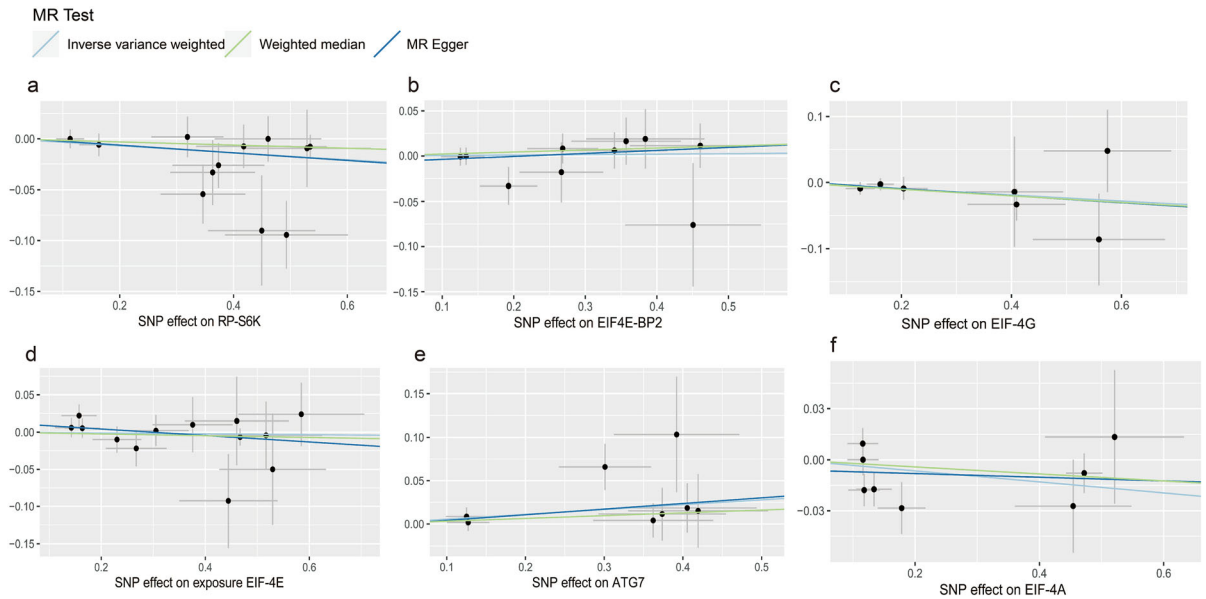

Fig. 3 As indicated by the intercept term of MR Egger's method, pleiotropy exists if the intercept term is significantly different from 0. The *slopes of the lines* represent the causal association, and each model has a separate line

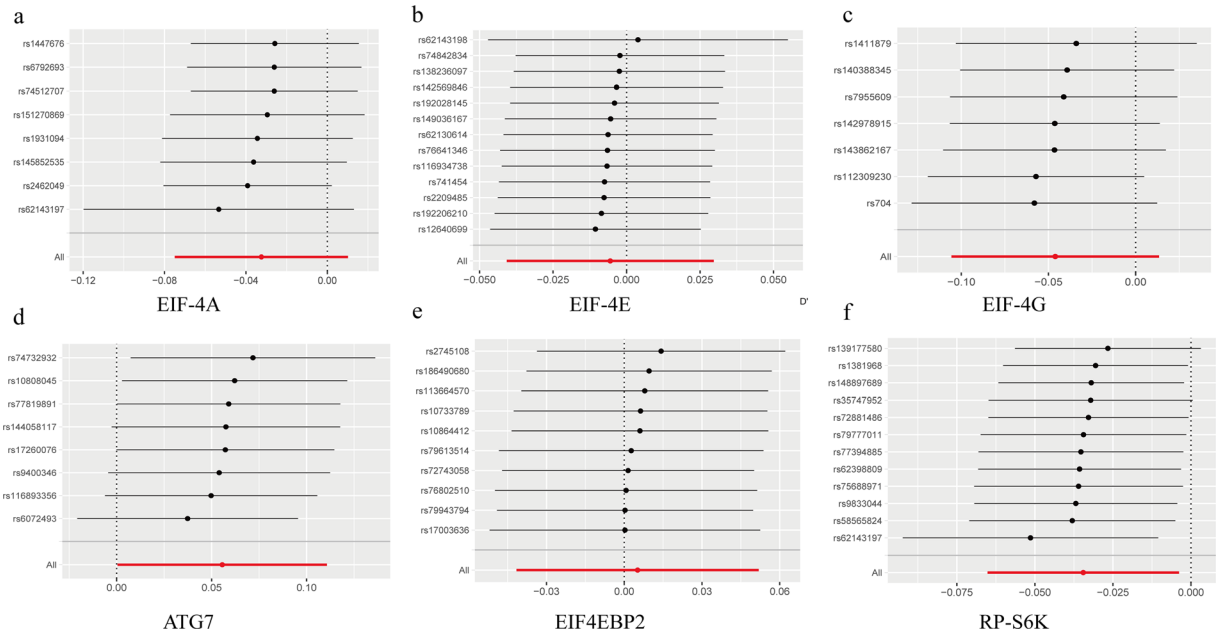

Fig. 4 Results of the leave-one-out method in MR sensitivity analysis. *RP-S6K* ribosomal protein-S6 kinase, *EIF4E-BP* eukaryotic translation initiation factor 4E-binding protein, *eIF4E* eukaryotic translation initiation factor 4E, *eIF4A* eukaryotic translation initiation factor 4A, *eIF4G* eukaryotic translation initiation factor 4G. Autophagy Related Protein 7

DISCUSSION

By performing MR analysis, our study estimated the causal relationship between several mTOR-related targets and RF risk. The data suggest that higher circulating levels of RP-S6K may be causally associated with lower RF risk, and higher circulating levels of ATG7 may be associated with higher RF risk, whereas EIF4EBP2, EIF-4E, EIF-4G, EIF-4A had no significant correlation with RF. Our study is the first to assess the risk between mTOR and RF using MR, and our findings reaffirm the possibility of mTOR in alleviating inflammatory autoimmune diseases.

In our study, RP-S6K was a protective factor of RF. RP-S6K, ribosomal protein S6 kinase, is a well-characterized target for mTORC1-mediated phosphorylation, which induces phosphorylation and activation of S6 to stimulate protein translation [18, 19]. mTOR plays a profound role in T cell lineage development. Specifically, it refers to promoting Th17 cell differentiation by S6K1 and S6K2. Mechanistically, S6K1 impinges on the downregulation of Gfi1, a negative regulator of Th17 differentiation. At the same time, S6K2 acts as a nuclear "carrier" for the transcription factor RORγ required for Th17 differentiation [9, 20]. (Fig. 5) Thus, mTOR may regulate T-cell metabolism by activating S6K and ultimately affect autoimmunity after *Streptococcus* infection.

ATG7 is considered to be a risk factor for RF. ATG7 is a ubiquitin-like ligase that forms classical bilayer-like autophagosomes, which play a vital role in the two ubiquitination modifications of autophagosomes formed by autophagic vesicles, occurring in the ATG5-ATG12 system and the LC3 system, respectively. Atg7 acts as E1 ubiquitin activase Atg12. Then, Atg12 is delivered to the E2 ubiquitin transferase Atg10. Finally, Atg12 binds to Atg5 to form a complex. After that, it is combined with Atg16L to form the ATG12-ATG5-ATG16L complex on the autophagosome's outer membrane, promoting the transfer of LC3 (Atg8) from Atg3 to the substrate phosphatidyl ethanolamine (PE). LC3 includes two interconvertible forms, LC3-I and LC3-II, which are involved in autophagosome membrane formation. Atg7 acts as an E1 enzyme to mediate the coupling of LC3-I to the

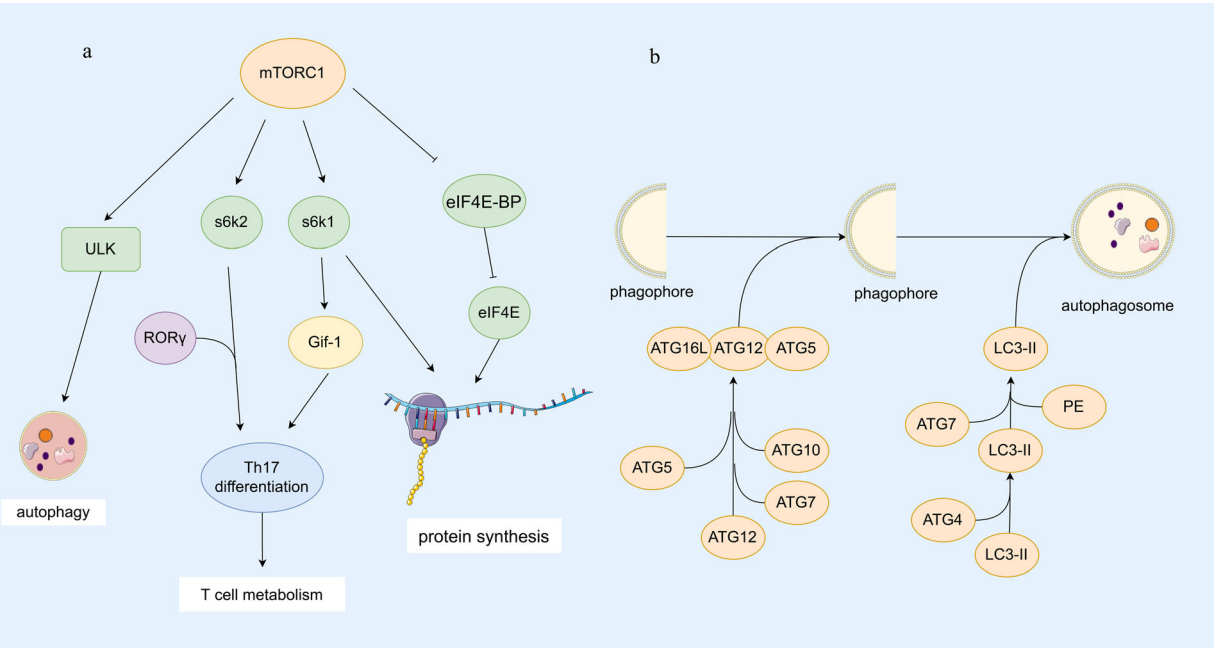

Fig. 5 **a** Signaling pathways downstream of mTORC1. **b** Schematic of the transformation of autophagic vesicles into autophagosomes. *ULK* A core protein in the autophagy signaling pathway, *s6k* ribosomal protein S6 kinase, *eIF4E-BP* eukaryotic translation initiation factor 4E-binding protein

substrate PE on the membrane surface of the autophagosome to form the membrane-bound form of LC3-II, which is a vital marker molecule of the autophagosome, which increases with the increase of the autophagosome membrane [21–23] (Fig. 5) Therefore, because of the characteristics of the autophagy mechanism, it is speculated that ATG7 affects autoimmunity, which in turn leads to RF after infection with *Streptococcus*. MTOR is involved in the induction and initiation of autophagy [24]. Several recent studies have confirmed that autophagy may be activated in mice inoculated with *Streptococcus* peptidoglycan polysaccharide, which appears to be consistent with increased expression of ATG7 autophagy initiation-related proteins. mTOR protein expression was lower in mice after rapamycin treatment than in mice vaccinated with streptococcal peptidoglycan polysaccharide [25]. In addition, Inomata et al. demonstrated that ATG7 is an essential component of the autophagy machinery, characterized by its independence from the members of the autophagic preinitiation complex. The occurrence of autophagy mediated by ATG7 has been observed in bone marrow-derived macrophages of streptococcal-infected mice [26]. Therefore, due to the characteristics of the autophagy mechanism, it is hypothesized that ATG7 affects RF after streptococcal infection.

Currently, RF is mainly treated by timely and appropriate antibiotic therapy for streptococcal A infection. This study demonstrates a causal relationship between mTOR and RF. Therefore, it may provide new ideas for developing related target drugs. MTOR inhibitors are effective immunosuppressive drugs for the prevention of acute rejection after solid organ transplantation [27]. They affect transcription and protein synthesis by integrating various signaling stimuli, and ultimately regulate cell apoptosis, growth and autophagy. Scientists have linked mTOR to various disease processes, such as neoplasia, arthritis, insulin resistance, and osteoporosis. Due to their inhibitory effects on cell growth and metabolic signaling pathways, they have recently been developed as antitumor-targeted therapies for various cancers [28]. Currently, two mTOR inhibitors (temsirolimus and everolimus) have been approved by the US Food and Drug Administration and the European Medicines Agency for the oncology market [29]. Because S6K isoforms play an essential role in the development and progression of several diseases, S6K isoforms are often considered potential therapeutic targets [30]. PF-4708671 is the first reported S6K1-specific inhibitor, which has only been reported to reduce the fibrotic area and apoptosis of cardiomyocytes [31]. The majority of mTOR-targeted therapies have been attributed to the inhibition of translation, and treatments are currently being investigated at this level of unregulated protein synthesis. Moreover, natural product-derived small molecule pomiferin triacetate as a novel mTORC1/C2 inhibitor has been identified to be effective at low micromolar concentrations and to efficiently attenuate translation [32]. mTOR, as the master switch of cell metabolism, is an adequate target protein for anticancer drugs. Therefore, many mTOR inhibitors are used as anticancer drugs in the clinic. However, resistance may develop, so continuously developing new allosteric mTOR inhibitors is necessary. Due to the high molecular weight and more chiral carbon of mTOR inhibitors, it is not easy to synthesize them. In the future, we can start with structural modification to improve their properties, develop new drug formulations, and carry out the treatment of other diseases [33]. Our study also provides insights into the development of mTOR inhibitors for the treatment of RF.

This study has several strengths. First, for the first time, MR analysis was employed to explore the causality of mTOR with RF, which could vastly reduce the influence of the environmental confounders and reverse causality, and be less prone to the bias of observational studies. However, several limitations should be considered when interpreting our findings. First, MR has only been analyzed through GWAS data, and this study lacks clinical trial verification. Therefore, we only speculate that mTOR and RF have a great possibility of correlation, which needs to be verified by follow-up experimental trials. Second, all GWAS data came from the European population. Whether our described findings would be consistent in other people remained to be investigated.

CONCLUSION

RP-S6K could be identified as a protective factor for RF, whereas ATG7 was a risk factor for RF. These findings may provide new insights into the intervention of RF.

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Data about the mTOR-related GWAS data was obtained from the INTERVAL study Proteomics-GWAS data. We thank all involved investigators for sharing their data. We want to acknowledge the participants and investigators of the Integrative Epidemiology Unit (IEU) GWAS database.

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Authorship. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Author Contributions. Yan-Fei Mu, Qian Wang, Jing-Xi Hu, Qi Wang, Yao-Chen Zhang, Ke-Yi Fan conceived and designed the analysis; Qian Wang, Jing-Xi Hu, Qi Wang, Yao-Chen Zhang, Ke-Yi Fan and Zi-Yi Han performed the analysis; Yan-Fei Mu and Qian Wang co-wrote and edited the manuscript; Sheng-Xiao Zhang, He-Yi Zhang, Ting Cheng, Rong Zhao, Shan Song, Jun Qiao and Cai-Hong Wang organized the review and revised the manuscript. All authors contributed to the article and approved the submitted version. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Disclosures. Yan-Fei Mu, Qian Wang, Jing-Xi Hu, Qi Wang, Yao-Chen Zhang, Ke-Yi Fan, Zi-Yi Han, He-Yi Zhang, Ting Cheng, Rong Zhao, Shan Song, Jun Qiao, Sheng-Xiao Zhang, Cai-Hong Wang have nothing to disclose.

Compliance with Ethics Guidelines. All the data used in this study were from public databases and did not require ethical approval. This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.

Data Availability. Data on RF outcomes were obtained publicly available from the Integrative Epidemiology Unit (IEU) GWAS database (https://gwas.mrcieu.ac.uk). Data about the mTOR-related GWAS data was obtained from the publicly available INTERVAL study Proteomics- GWAS data, which quantified 3,622 plasma proteins in 3,301 healthy participants.

REFERENCES

1. Karthikeyan G, Guilherme L. Acute rheumatic fever. Lancet (London, England). 2018;392(10142):161–74.

2. Carapetis JR, Beaton A, Cunningham MW, Guilherme L, Karthikeyan G, Mayosi BM, et al. Acute rheumatic fever and rheumatic heart disease. Nat Rev Dis Primers. 2016;2:15084.

3. Marijon E, Mirabel M, Celermajer DS, Jouven X. Rheumatic heart disease. Lancet (London, England). 2012;379(9819):953–64.

4. Watkins DA, Zuhlke LJ, Engel ME, Mayosi BM. Rheumatic fever: neglected again. Science (New York, NY). 2009;324(5923):37.

5. Belay W, Aliyu MH. Rheumatic heart disease is missing from the global health agenda. Ann Glob Health. 2021;87(1):110.

6. Saxton RA, Sabatini DM. mTOR signaling in growth, metabolism, and disease. Cell. 2017;168(6): 960–76.

7. Suto T, Karonitsch T. The immunobiology of mTOR in autoimmunity. J Autoimmun. 2020;110: 102373.

8. Soltani A, Bahreyni A, Boroumand N, Roshan MK, Khazaei M, Ryzhikov M, et al. Therapeutic potency of mTOR signaling pharmacological inhibitors in the treatment of proinflammatory diseases, current status, and perspectives. J Cell Physiol. 2018;233(6): 4783–90.

9. Wyman B, Perl A. Metabolic pathways mediate pathogenesis and offer targets for treatment in rheumatic diseases. Curr Opin Rheumatol. 2020;32(2):184–91.

10. Perl A. Activation of mTOR (mechanistic target of rapamycin) in rheumatic diseases. Nat Rev Rheumatol. 2016;12(3):169–82.

11. Kim YC, Guan KL. mTOR: a pharmacologic target for autophagy regulation. J Clin Investig. 2015;125(1):25–32.

12. Liu E, Perl A. Pathogenesis and treatment of autoimmune rheumatic diseases. Curr Opin Rheumatol. 2019;31(3):307–15.

13. Sun BB, Maranville JC, Peters JE, Stacey D, Staley JR, Blackshaw J, et al. Genomic atlas of the human plasma proteome. Nature. 2018;558(7708):73–9.

14. Pierce BL, Ahsan H, Vanderweele TJ. Power and instrument strength requirements for Mendelian randomization studies using multiple genetic variants. Int J Epidemiol. 2011;40(3):740–52.

15. Kamat MA, Blackshaw JA, Young R, Surendran P, Burgess S, Danesh J, et al. PhenoScanner V2: an expanded tool for searching human genotype-phenotype associations. Bioinform (Oxford, England). 2019;35(22):4851–3.

16. Burgess S, Bowden J, Fall T, Ingelsson E, Thompson SG. Sensitivity analyses for robust causal inference from mendelian randomization analyses with multiple genetic variants. Epidemiology. 2017;28(1):30–42.

17. Bowden J, Del Greco MF, Minelli C, Davey Smith G, Sheehan NA, Thompson JR. Assessing the suitability of summary data for two-sample Mendelian randomization analyses using MR-Egger regression: the role of the I2 statistic. Int J Epidemiol. 2016;45(6):1961–74.

18. Weichhart T, Hengstschläger M, Linke M. Regulation of innate immune cell function by mTOR. Nat Rev Immunol. 2015;15(10):599–614.

19. Powell JD, Pollizzi KN, Heikamp EB, Horton MR. Regulation of immune responses by mTOR. Annu Rev Immunol. 2012;30:39–68.

20. Huang H, Long L, Zhou P, Chapman NM, Chi H. mTOR signaling at the crossroads of environmental signals and T-cell fate decisions. Immunol Rev. 2020;295(1):15–38.

21. Szwed A, Kim E, Jacinto E. Regulation and metabolic functions of mTORC1 and mTORC2. Physiol Rev. 2021;101(3):1371–426.

22. Alers S, Löffler AS, Wesselborg S, Stork B. Role of AMPK-mTOR-Ulk1/2 in the regulation of autophagy: cross talk, shortcuts, and feedbacks. Mol Cell Biol. 2012;32(1):2–11.

23. Codogno P, Mehrpour M, Proikas-Cezanne T. Canonical and non-canonical autophagy: variations on a common theme of self-eating? Nat Rev Mol Cell Biol. 2011;13(1):7–12.

24. Cayo A, Segovia R, Venturini W, Moore-Carrasco R, Valenzuela C, Brown N. mTOR activity and autophagy in senescent cells, a complex partnership. Int J Mol Sci. 2021;22(15):8149.

25. Xie D, Zhao T, Zhang X, Kui L, Wang Q, Wu Y, et al. Autophagy contributes to the rapamycin-induced improvement of otitis media. Front Cell Neurosci. 2021;15: 753369.

26. Inomata M, Xu S, Chandra P, Meydani SN, Takemura G, Philips JA, et al. Macrophage LC3-associated phagocytosis is an immune defense against Streptococcus pneumoniae that diminishes with host aging. Proc Natl Acad Sci USA. 2020;117(52): 33561–9.

27. Zoncu R, Efeyan A, Sabatini DM. mTOR: from growth signal integration to cancer, diabetes and ageing. Nat Rev Mol Cell Biol. 2011;12(1):21–35.

28. Zou Z, Tao T, Li H, Zhu X. mTOR signaling pathway and mTOR inhibitors in cancer: progress and challenges. Cell Biosci. 2020;10:31.

29. Vergès B, Cariou B. mTOR inhibitors and diabetes. Diabetes Res Clin Pract. 2015;110(2):101–8.

30. Magnuson B, Ekim B, Fingar DC. Regulation and function of ribosomal protein S6 kinase (S6K) within mTOR signalling networks. Biochem J. 2012;441(1):1–21.

31. Pearce LR, Alton GR, Richter DT, Kath JC, Lingardo L, Chapman J, et al. Characterization of PF-

4708671, a novel and highly specific inhibitor of p70 ribosomal S6 kinase (S6K1). Biochem J. 2010;431(2):245–55.

32. Bajer MM, Kunze MM, Blees JS, Bokesch HR, Chen H, Brauss TF, et al. Characterization of pomiferin triacetate as a novel mTOR and translation inhibitor. Biochem Pharmacol. 2014;88(3):313–21.

33. Chen Y, Zhou X. Research progress of mTOR inhibitors. Eur J Med Chem. 2020;208: 112820.