Statin safety among Chinese adolescents: a Mendelian randomisation study (abridged secondary publication)

SL Au Yeung *, HS Lam, YH Chan, CM Schooling

KEY MESSAGES

1. There is limited evidence on the long-term safety of statin use among adolescents.
2. As a form of genetic validation, this Mendelian randomisation study used randomly allocated variants within the HMGCR protein-encoding gene (leading to reductions in low-density lipoprotein cholesterol) to infer statin safety based on data from the Hong Kong 'Children of 1997' birth cohort.
3. Genetic evidence did not suggest substantial safety concerns about statins among Chinese adolescents.
4. This proof-of-concept study used a well-characterised birth cohort to infer the safety of drug targets among adolescents.

Hong Kong Med J 2024;30(Suppl 3):S11-5
HMRF project number: 07181916

[1] SL Au Yeung, [2] HS Lam, [3] YH Chan, [1,4] CM Schooling

[1] *School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China*
[2] *Department of Paediatrics, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China*
[3] *Division of Cardiology, Department of Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China*
[4] *Graduate School of Public Health and Health Policy, City University of New York, New York, United States*

* Principal applicant and corresponding author: ayslryan@hku.hk

Introduction

Three-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) inhibitors, collectively referred to as statins, are the first-line pharmacologic treatments for children with familial hypercholesterolaemia (FH); these inhibitors have demonstrated reductions in cardiovascular risk in adulthood. However, as obesity rates increase among adolescents, particularly in East Asian populations, statin prescriptions may be needed to mitigate the risk of atherosclerotic cardiovascular disease in non-FH adolescents who have elevated and uncontrolled lipid profiles. However, there has been a lack of safety studies regarding these medications in adolescents. It is in the public health interest to evaluate any adverse effects of statins in adolescents.

Real-world data, such as pharmaco-epidemiologic studies, are susceptible to confounding based on indications or immortal time bias. Mendelian randomisation studies have been used to investigate the adverse effects of medications, such as anti-hypercholesteremia and antihypertensive medications, in European adult populations. Here, we evaluated the association of genetically proxied inhibition of HMGCR with safety outcomes in a population-representative birth cohort in Hong Kong.

Methods

This Mendelian randomisation study had three instrumental variable assumptions. First, to ensure relevance, genetic variants within the *HMGCR* protein-encoding gene exhibiting associations with low-density lipoprotein cholesterol (LDL-C) were regarded as proxies for statin use. Second, the study assumed that genetic variants could not be confounded (eg, population stratification), ensuring independence. Third, the study assumed that genetic variants were independent of exposure-related outcomes, thereby addressing the exclusion restriction.[1]

The Hong Kong 'Children of 1997' birth cohort is a population-representative Chinese birth cohort that recruited 88% of all ethnic Chinese births (n=8327) between April and May 1997.[2] Recruitment was conducted at all maternal and children health centres in Hong Kong during the first postnatal visit for free preventive care and immunisations. Infant and family characteristics were recorded using a self-administered questionnaire. In 2005, record linkage was established to obtain routine information and clinical measurements (96% successful matching, n=7999). During the Biobank Clinical follow-up phase 1 in 2013 to 2016 and the supplementary Biobank Clinical follow-up phase 2 in 2017, 3460 and 158 participants, respectively, provided biospecimens (blood, saliva, urine, stool, hair, and nails) and completed comprehensive measurements (eg, anthropometrics).

Fasting blood samples and their derivatives (eg, buffy coat and plasma) were used for biochemical assays such as liver function tests. DNA was extracted from blood, buffy coat, or saliva samples; genotyping

was performed for 3582 participants. Phasing and genotyping imputation were conducted. For quality control, samples with a call rate <0.98, recorded sex not matching genetically inferred sex, second-degree relatedness or above, high heterozygosity (>3 standard deviations), or variants with a call rate <0.98 and imputation score <0.3 were excluded.

We identified genetic instruments to proxy the effects of HMGCR inhibitors using the Global Lipids Genetics Consortium, a genome-wide association study that included 188 577 middle-aged participants of predominantly European ancestry.[3] Six genetic variants (rs12916, rs17238484, rs5909, rs2303152, rs10066707, and rs2006760) are located within 100 kilobase pairs on either side of the *HMGCR* protein-encoding gene and associated with LDL-C at a genome-wide significance level ($P<5\times10^{-8}$).

To assess the validity of these variants in an East Asian population, we cross-verified the variant-LDL-C associations in the 'Children of 1997' birth cohort, with adjustments for age, sex, and the top six principal components of ancestry. We selected variants associated with LDL-C that displayed low linkage disequilibrium (clustering $r^2<0.3$, clustering window of 10 000 kilobases) in a reference panel of East Asian ancestry from the 1000 Genomes Project (Phase 3).

To maximise statistical power, an externally weighted genetic risk score for HMGCR was constructed for each participant. Specifically, the genetic risk score for HMGCR was constructed by summing the number of all LDL-C-lowering alleles for each variant in the *HMGCR* gene region, weighted according to the effect of each variant on LDL-C.[3] As in a previous study,[4] participants were first categorised using the median HMGCR score as proxies for statin use (participants with < median HMGCR score) and placebo (participants with ≥ median HMGCR score); they were subsequently categorised using the quartiles of HMGCR scores to assess potential dose-response relationships.

The outcomes included lipid profile (LDL-C, high-density lipoprotein cholesterol, and triglycerides), glycaemic traits (fasting glucose and glycated haemoglobin), liver function (alkaline phosphatase, alanine transaminase, albumin, and bilirubin) assessed at ~17.6 years during the Biobank clinical follow-up, and Tanner stage (breasts for girls and genitals for boys) and Tanner stage (public hair) at ~11 years.

The associations of HMGCR scores and potential confounders (sex, breastfeeding duration, highest parental education, housing type, and physical activity) were assessed using the Chi-squared test and analysis of variance. The associations of categorical HMGCR scores with outcomes were determined using multivariable linear and logistic regression, with adjustments for age, sex, and the top six principal components of ancestry. To preclude the possibility of false-positives due to inclusion of correlated variants, we also repeated the analysis using the index variant (rs12916) of *HMGCR* as a sensitivity analysis. The P value threshold after correction for multiple tests was set to 5×10^{-3} ($\alpha=0.05/10$).

Results

There were 1753 male and 1669 female participants with both valid genetic data and LDL-C measurements (Table). Two (rs12916 and rs17238484, LD $r^2=0.36$) of the six variants were selected to derive HMGCR scores, which were associated with LDL-C (-0.57 mmol/L, 95% confidence interval [CI]= -0.31 to -0.84). The HMGCR score was not associated with any of the potential confounders considered, other than parental education.

Compared with the reference group (participants with ≥ median HMGCR score), participants with < median HMGCR score had lower LDL-C (beta= -0.09 mmol/L, 95% CI= -0.13 to -0.04). In the dose-response analysis, lower HMGCR quartiles were associated with a stepwise decrease in LDL-C. In the sensitivity analysis, a T-allele increase in the HMGCR index variant rs12916 was associated with lower LDL-C (-0.07, 95% CI= -0.10 to -0.04). The HMGCR score was not associated with other lipid traits. For other outcomes, there were no associations of HMGCR scores below the median with glycaemic traits, liver function at age ~17.5 years, or Tanner stage (breasts for girls and genitals for boys) and Tanner stage (public hair) at age ~11 years. Sensitivity analyses yielded consistent findings, except that some HMGCR categories were nominally associated with lower glycated haemoglobin and lower albumin (Fig).

Discussion

The effect of HMGCR on LDL-C reduction was similar to the results of previous randomised controlled trials and Mendelian randomisation studies involving European and East Asian adults. A systematic review showed that short-term statin use (<48 weeks) among paediatric patients with FH was relatively safe.[5] Our study provides genetic evidence that long-term on-target effects of statins do not adversely affect glycaemic traits, liver function, or pubertal development in Chinese adolescents.

Consistent with previous studies, we did not find an association between genetic inhibition of HMGCR and liver function, although inadequate statistical power may explain the null findings. The rare statin-associated asymptomatic increase in transaminases (>3 times the upper limit of normal) and hepatotoxicity reported in clinical trials are likely due to idiosyncratic or immune

TABLE. Characteristics of participants in the 'Children of 1997' birth cohort according to 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) score

Variable	No. of participants	HMGCR score < median (n=1711)*	HMGCR score ≥ median (n=1711)*	P value
Sex	3422			0.43
Male		888/1711 (51.9)	865/1711 (50.6)	
Female		823/1711 (48.1)	846/1711 (49.4)	
Breastfeeding duration	3365			0.49
Never		882/1679 (52.5)	912/1686 (54.1)	
Partial or exclusive <3 months		664/1679 (39.6)	656/1686 (38.9)	
Exclusive ≥3 months		133/1679 (7.9)	118/1686 (7.0)	
Highest parental education level	3400			0.03
Grade 9 or below		483/1697 (28.5)	504/1703 (29.6)	
Grade 10-11		709/1697 (41.8)	762/1703 (44.8)	
Grade 12 or above		505/1697 (29.7)	437/1703 (25.7)	
Housing type at birth	3312			0.33
Home ownership		271/1643 (16.5)	269/1669 (16.1)	
Private flat		677/1643 (41.2)	652/1669 (39.1)	
Public/squatter/other		695/1643 (42.3)	748/1669 (44.8)	
Screen time, h	2324	4.87±2.67	4.84±2.47	0.62
Physical activity, h	1669	5.95±3.02	5.87±2.48	0.92
Low-density lipoprotein, mmol/L	3422	2.10±0.66	2.19±0.65	<0.001
High-density lipoprotein, mmol/L	3422	1.55±0.34	1.55±0.34	0.76
Triglycerides, log_{10}mmol/L	3422	-0.14±0.17	-0.13±0.18	0.63
Fasting glucose, mmol/L	3334	4.64±0.34	4.64±0.35	0.53
Glycated haemoglobin, %	3412	5.40±0.25	5.39±0.26	0.25
Albumin, g/L	3422	47.40±2.65	47.36±2.59	0.93
Bilirubin, log_{10}μmol/L	3180	1.06±0.20	1.05±0.20	0.39
Alkaline phosphatase, log_{10}IU/L	3422	1.89±0.14	1.89±0.14	0.65
Alanine transaminase, log_{10}IU/L	3099	1.24±0.19	1.24±0.19	0.49
Tanner stage (breasts/genitals)	918			0.76
1		181/468 (38.7)	174/450 (38.7)	
2		126/468 (26.9)	121/450 (26.9)	
3		124/468 (26.5)	116/450 (25.8)	
4		28/468 (6.0)	34/450 (7.6)	
5		9/468 (1.9)	5/450 (1.1)	
Tanner stage (pubic hair)	872			0.76
1		335/445 (75.3)	326/427 (76.4)	
2		77/445 (17.3)	64/427 (15.0)	
3		22/445 (4.9)	25/427 (5.9)	
4		11/445 (2.5)	11/427 (2.6)	
5		0/445 (0)	1/427 (0.2)	

* Data are presented as mean±standard deviation or No. (%) of participants

allergic reactions; in 2012, the US Food and Drug Administration recommended removal of routine periodic monitoring of liver enzymes among statin users.

This study had some limitations. First, the study was not able to assess pharmacological interventions administered at specific time points or doses, or the effects on individuals with particular indications (eg, FH), which is a common limitation of drug target Mendelian randomisation studies. Second, although

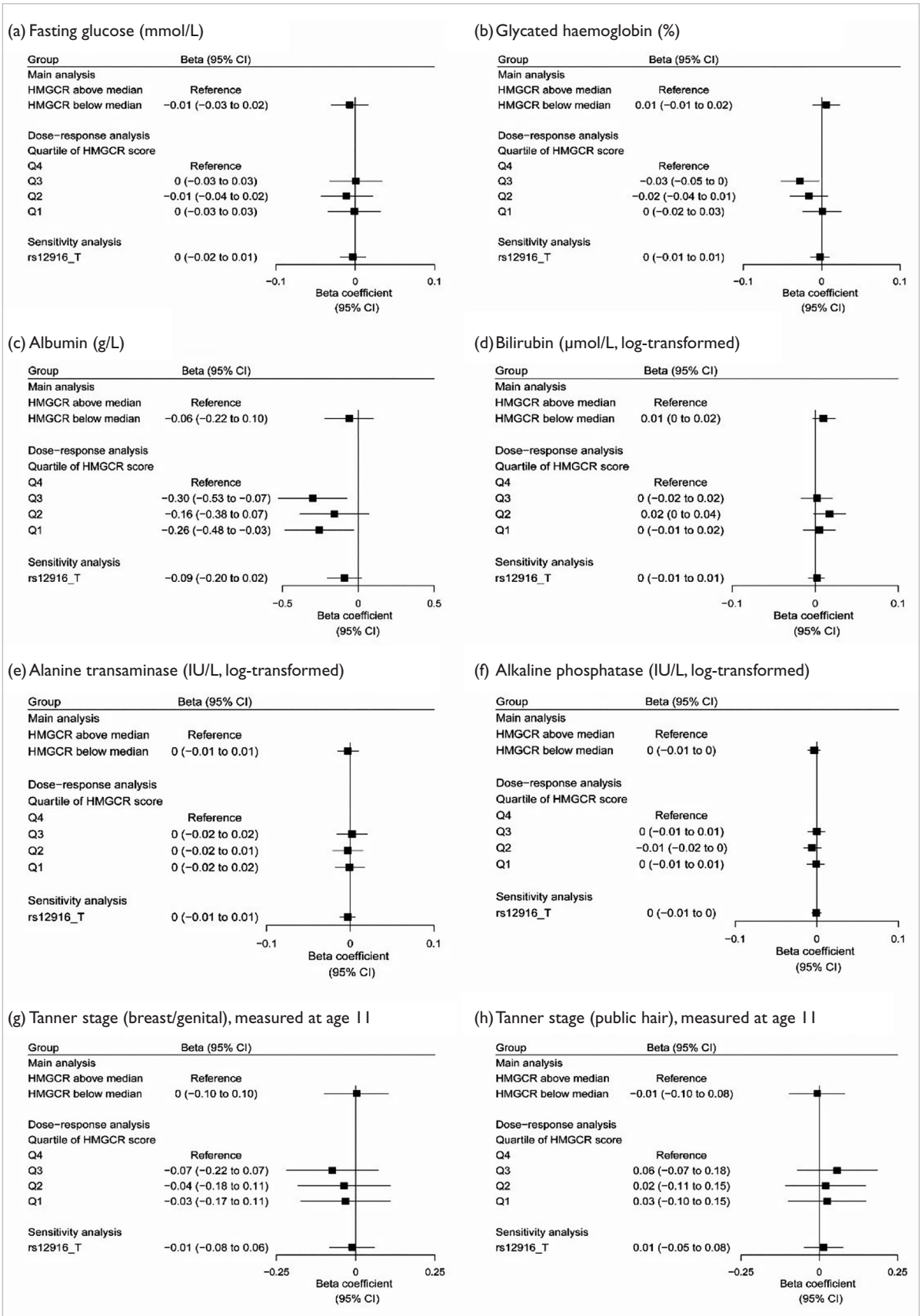

FIG. Association of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) low-density lipoprotein cholesterol score categories with (a) fasting glucose, (b) glycated haemoglobin, (c) albumin, (d) bilirubin, (e) alanine transaminase, (f) alkaline phosphatase, (g) Tanner stage (breasts/genitals), and (h) Tanner stage (pubic hair) in the 'Children of 1997' birth cohort.
Abbreviation: 95% CI = 95% confidence interval

the genetic variants for HMGCR inhibition were selected in people of European descent and validated in people of East Asian descent, future investigations should use ethnicity-specific genome-wide association studies to generate ethnicity-specific HMGCR variants. Third, because of sample size and statistical power constraints, we were unable to consider sex-specific associations and exclude the possibility of false-negatives, even though this is one of the largest studies in Chinese adolescents to date. Finally, we did not include all possible adverse effects such as muscle symptoms and related biomarkers. A broader spectrum of potential adverse effects should be assessed in larger studies that link electronic health records for clinical outcome with Biobank studies (eg, the All of Us study,[6] which recruited more than 40 000 participants aged 18 to 29 years).

Conclusion

This study did not yield evidence to suggest substantial concerns about long-term statin safety in Chinese adolescents. This proof-of-concept study showed the use of well-characterised birth cohorts with genetic and phenotypic data to facilitate assessments of drug target efficacy and safety among adolescents.

Funding

This study was supported by the Health and Medical Research Fund, Health Bureau, Hong Kong SAR Government (#07181916). The full report is available from the Health and Medical Research Fund website (https://rfs2.healthbureau.gov.hk). The 'Children of 1997' birth cohort was initially supported by the Health Care and Promotion Fund (#216106) and then by the Health and Health Services Research Fund (#03040771, 05060671, 07080751, and 07080841) and the Research Fund for the Control of Infectious Diseases (#04050172, 06060592). The birth cohort has also received funding from the University Research Committee Strategic Research Theme of Public Health Granted Research, The University of Hong Kong. The most recent follow-up was partly funded by the WYNG Foundation. The GWAS analyses was funded by the Health and Medical Research Fund (CFS-HKU1).

Disclosure

The results of this research have been previously published in:

1. Luo S, Lam HS, Chan YH, et al. Assessing the safety of lipid-modifying medications among Chinese adolescents: a drug-target Mendelian randomization study. BMC Med 2023;21:410.

Acknowledgements

We would like to thank the Global Lipids Genetics Consortium for providing genome-wide association summary statistics. We thank the participants and their parents of the 'Children of 1997' birth cohort.

References

1. Schmidt AF, Finan C, Gordillo-Maranon M, et al. Genetic drug target validation using Mendelian randomisation. Nat Commun 2020;11:3255.
2. Schooling CM, Hui LL, Ho LM, Lam TH, Leung GM. Cohort profile: 'children of 1997': a Hong Kong Chinese birth cohort. Int J Epidemiol 2012;41:611-20.
3. Willer CJ, Schmidt EM, Sengupta S, et al. Discovery and refinement of loci associated with lipid levels. Nat Genet 2013;45:1274-83.
4. Ference BA, Robinson JG, Brook RD, et al. Variation in PCSK9 and HMGCR and risk of cardiovascular disease and diabetes. N Engl J Med 2016;375:2144-53.
5. Dombalis S, Nash A. The effect of statins in children and adolescents with familial hypercholesterolemia: a systematic review. J Pediatr Health Care 2021;35:292-303.
6. National Institutes of Health. Data snapshots of All of Us research program. Accessed May 2023. Available from: https://www.researchallofus.org/data-tools/data-snapshots/.