

Original Research

Differential associations of statin treatment and polymorphism in genes coding for HMGCR and PCSK9 to risk for insomnia

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1. Abstract

Importance: Statins have been linked to an increased risk for insomnia, but the literature is controversial. Moreover, it is unknown, if the potential effects are directly related to the inhibition of the statin target HMGCR, the subsequently lowered cholesterol levels, or other off-target

effects of statins. **Aims:** To investigate the association of statin treatment and genetic proxies of cholesterol lowering drugs with the risk for insomnia and chronotype in a large population-based cohort. **Methods:** A cross-sectional cohort study based on baseline data collected between 2006–2010 in UK biobank cohort was conducted. European participants without any history of psychiatric/neurological

disorders or of stroke and with available genetic data as well as information on statin use were included in the present study. Self-reported measures of insomnia and chronotype were analysed (a) in statin users versus control subjects, (b) subjects carrying single nucleotide polymorphisms (SNPs) in the *HMGCR* gene, which are associated with reduced enzymatic function and lower cholesterol levels (rs17238484 and rs12916) and (c) subjects carrying a SNP in the *PCSK9* gene (rs1159147), which leads to lower cholesterol levels independent of *HMGCR*. The main analysis were performed using multivariable regression models. Statin treatment and SNPs in *HMGCR* and *PCSK9* genes were used as exposures and main outcomes were insomnia and chronotype. **Results:** A total of 206,801 participants (mean [SD] age, 57.5 [7.9] years; 56% women; 20% statin users) were included in the present study. Statin users had an increased risk of insomnia compared to controls (odds ratio [95% CI], 1.07 [1.03 to 1.11]; $p = 1.42 \times 10^{-4}$). A similar effect was observed for *PCSK9* rs1159147-T allele (1.07 [1.01–1.14]; 0.014), while the two gene variants of *HMGCR* were associated with a reduced risk for insomnia (rs17238484-G: 0.97 [0.95 to 0.99]; 0.014 and rs12916-T: 0.97 [0.96 to 0.99]; 0.002). In regard to chronotype, there was no effect of either statin treatment or *HMGCR* SNPs, but the *PCSK9* rs1159147-T allele was associated with a higher evening preference (1.17 [1.06 to 1.29]; 0.001). **Conclusion:** Our data suggests that statin treatment can pose an increased risk for insomnia in the European population. Interestingly, there was no agreement between the effects of statins and the effects of reduced *HMGCR* activity based on genetic variants, suggesting that the observed unfavourable effect of statins on sleep is conveyed through other targets. This further explains why the literature on statin effects on sleep is not conclusive. Finally our data encourage further investigations into the molecular processes linking statins, *HMGCR* and *PCSK9* to sleep behaviour.

2. Introduction

Hypercholesterolemia is a major causative risk factor for cardiovascular diseases (CVDs) [1], which in turn are the primary cause of death around the world (WHO). The main drug target to counteract hypercholesterolemia is the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (*HMGCR*), which catalyses the rate-limiting step in cholesterol biosynthesis [2]. Statin drugs inhibit *HMGCR* enzyme activity, which leads to a reduction of cholesterol synthesis in the liver by increasing low-density lipoprotein (LDL) receptor expression at the surface of the liver cells, resulting in increased uptake of LDL from the blood and ultimately decreased plasma concentrations of LDL- and other apolipoprotein B-containing lipoproteins. This, in turn, leads to an effective reduction in primary and secondary CVDs [2, 3]. As a result of increased prevalence of CVDs and higher efficiency of treatment, statin drugs

have become one of the most widely prescribed medication worldwide. In the US, every fourth adult aged over 40 years is receiving statins, and an estimated 56 million people are eligible for statin treatment based on new guidelines [4].

A second class of effective drugs for CVD protection are the so-called PCSK9 inhibitors (i.e., alirocumab and evolocumab) [5, 6]. PCSK9 inhibitors lower plasma cholesterol by preventing the protein Proprotein convertase subtilisin/kexin type 9 (PCSK9) from attaching to LDL receptors [7, 8]. This enables the recycling of LDL receptors, increases the uptake of LDL particles into cells and thereby reduces circulating LDL particles.

In the existing literature, sleep disturbances, including symptoms of insomnia, have been linked to the treatment with cholesterol-lowering drugs in general and with statin treatment in particular [9–11]. Insomnia is a frequent complaint in the general practice, and epidemiological studies show that the prevalence of insomnia symptoms is about 30–35% in the global population [12]. Depending on the severity, insomnia can be a serious condition with large impact on quality of life [12–14]. There are many predisposing factors for insomnia, including genetic factors and the use of medication [12], and insomnia symptoms experienced as side effects of drug treatment may significantly affect the probability of a patient adhering to treatment [15]. These clinical considerations underscore the urgency to identify the association between insomnia and a given drug.

However, with regard to statin treatment and sleep disturbances, literature remains inconclusive as the results of previous studies have been highly inconsistent [10]. These inconsistent findings might relate to a range of different factors. First, major side effects are difficult to identify in randomized controlled trials (RCTs) due to the usually relatively small size of the study population [11, 16, 17]. In addition, only few studies have investigated sleep disturbances as a primary outcome [18–20]. Furthermore, there is a range of factors that tend to vary across studies, including statin type [21], patient age [22], study type and statistical analysis [23] which might all influence the study outcome. Finally, it is known that statins not only interact with their primary target *HMGCR* (on target), but also directly interact with other proteins as well as membranes (off target effects) [24–26]. Little is known so far about the contribution of on-target versus off-target effects of statin treatment, which would aid in understanding the drugs' side effects as well as controversial study outcomes.

A way to overcome many of the aforementioned confounding factors and gain better insight into actual on-target effects is to perform genetic association studies that evaluate the effect of different functional gene variants on specific phenotypes. Using this approach, a long-suspected link between both statins and PCSK9 inhibitors on diabetes measures (e.g., plasma cholesterol, body weight, waist circumference, plasma insulin and glucose concentrations)

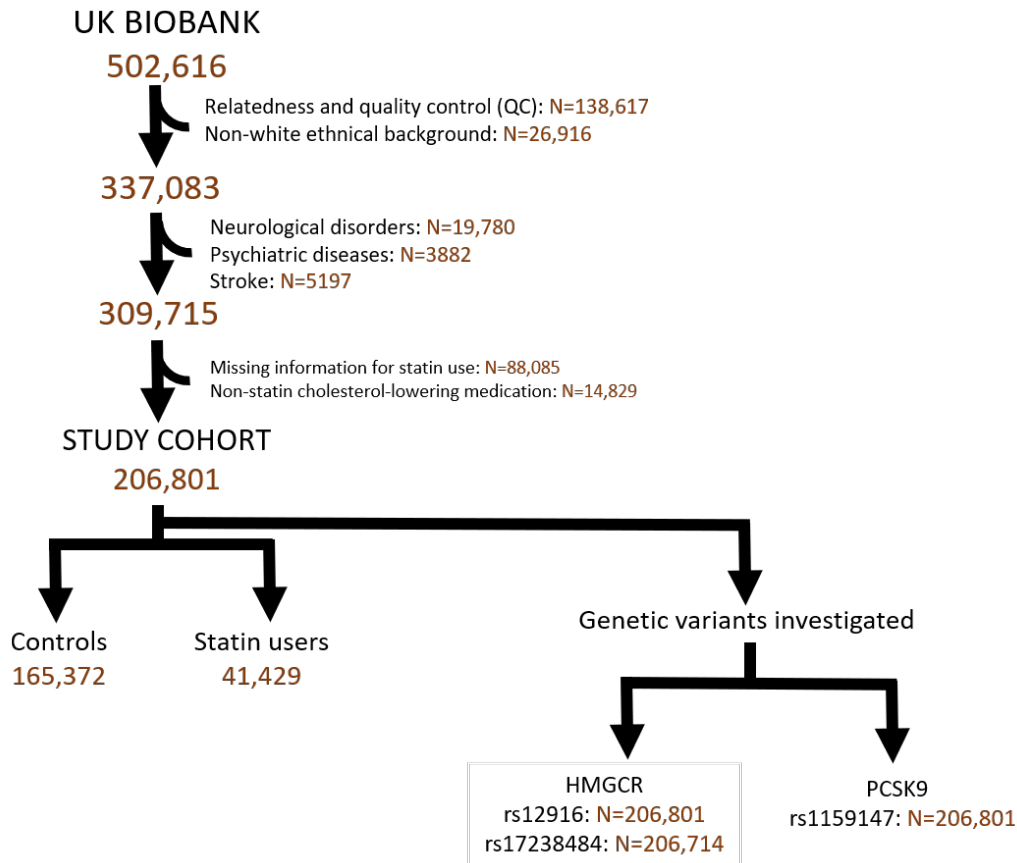


Fig. 1. Study population.

has been confirmed by studying the association of single-nucleotide polymorphisms (SNPs) in the two respective target genes, *HMGCR* and *PCSK9* [27, 28]. Thus, by using the SNPs as proxies for lowering cholesterol levels directly related to the primary targets of these two drug classes, potential on-target side effects of drug treatment can be identified by exploring their molecular inhibition.

Following this rationale, our work aimed to shed light on the controversial connection of statins and sleep disturbances by investigating a possible relationship between statin treatment, on the one hand, and SNPs in the *HMGCR* and *PCSK9* genes, on the other hand, and the risk for insomnia as well as the individual chronotype in a large European population.

3. Materials and methods

3.1 Ethics

This study used the UK Biobank resource (<http://www.ukbiobank.ac.uk/>) to analyse data that has previously been collected. The UK Biobank study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All participants in the UK Biobank study

have given informed consent for data collection, data storage and subsequent data analysis. Ethical permission for our study was given by the responsible local authorities, *Regionala Etikprövningsnämnden* (now *Etikprövningsmyndigheten*, Swedish Ethical Review Authority, EPN), Uppsala, under registration number (*Diarienummer*, Dnr) 2017/198.

3.2 UK Biobank data

UK Biobank is an open access resource with a collection of longitudinal health-related data and biological samples [29]. It constitutes a prospective study of more than 500,000 participants aged between 40 and 69 years when attending the assessment centre, and living in the United Kingdom. Present study is based on baseline data, collected between 2006 and 2010. Generally, detailed information regarding all variables included in the study can be found on the UK Biobank website (<http://www.ukbiobank.ac.uk/>). Specifically, we extracted data on the following parameters at baseline: insomnia (UK Biobank Field ID (FID): 1200), chronotype (FID: 1180), age (FID: 21003), sex (FID: 31), total cholesterol level and LDL-cholesterol level (FID: 30690), body mass index (BMI) (FID 21001), education (FID: 6138), alcohol intake frequency (FID: 1558), sleep duration (FID: 1160), smoking status (FID: 20116), physi-

cal activity (FID: 884), diagnosis of diabetes (FID: 2443), diagnosis of hypertension, angina and heart attack (ID: 6150), use of hypertensive medications, statins and insulin (FID: 20003) and Townsend deprivation index (TDI) reflecting socio-economic status of the participant based on area of residence at the time of recruitment (FID: 189).

3.3 Exclusion criteria

In the current analyses, participants with non-white ethnical background ($n = 26,916$), genetic relatedness and quality-control failure of the genotyped data ($n = 138,617$) were excluded (Fig. 1). Moreover, all the participants with neurological ($n = 19,916$) or psychiatric disorders ($n = 3882$), or persons who have had a stroke ($n = 5197$) were excluded to minimize confounding due to these factors. Following these exclusions, 206,801 participants with genetic data as well as information on medication use were included in the present study (Fig. 1). The study participants were then subjected to two independent analyses, i.e., non-genetic and genetic. In non-genetic analyses, statin medication was used as exposure while in genetic analyses, genetic variants were used as proxies for statin use.

3.4 Single-nucleotide polymorphisms

We analysed three SNPs (two in the *HMGCR* gene (rs17238484 and rs12916) and one in the *PCSK9* gene (rs11591147)) that are known to be linked to cholesterol lowering and can be used as proxies for statin use and *PCSK9* inhibition respectively [28, 30]. We confirmed that all SNPs were in Hardy-Weinberg equilibrium.

3.5 Statin treatment

Use of statin medication was self-reported, and only concerned medication that is regularly taken. The prescribed statin drugs present in the UK Biobank cohort were simvastatin, atorvastatin, fluvastatin, rosuvastatin and pravastatin. However, majority of the statin users (93%) were taking lipophilic statins (i.e., atorvastatin, simvastatin and fluvastatin) and very few (7%) were taking hydrophilic statins (i.e., rosuvastatin, pravastatin). Moreover, of the lipophilic statin users, 79.0% used simvastatin, 20.7% used atorvastatin and only 0.3% used fluvastatin at the first assessment in the UK Biobank cohort.

3.6 Insomnia

Information regarding insomnia symptoms had been collected as part of a touch-screen questionnaire. The participants had to answer the question “Do you have trouble falling asleep at night, or do you wake up in the middle of the night?” by choosing one of the three semi-quantitative options: (1) “never/rarely”, (2) “sometimes” or (3) “usually”. For our study, we recoded this information into a binary variable by grouping together “never/rarely” and “sometimes” and treated them as controls as done previously [31], while participants reporting “usually” were treated as those having insomnia.

3.7 Chronotype

Information regarding chronotype was collected through a question at baseline by asking “Do you consider yourself to be?” and the participants can choose from the answers “definitely a ‘morning’ person”, “more a ‘morning’ than an ‘evening’ person”, “more an ‘evening’ than a ‘morning’ person” or “definitely an ‘evening’ person”.

3.8 Covariates

The analysis included a set of covariates, which were chosen based on their link to sleep behaviour and cardiovascular health [31, 32] (Supplementary Table 1). In brief, age (years), sex (male/female), BMI ($\text{weight}/(\text{height})^2$, calculated from the measurement of body weight (kg) and height (cm)), physical activity (number of days/week of moderate physical activity 10+ minutes; self-reported), sleep duration (hours/day; self-reported), total cholesterol and LDL-cholesterol levels (mmol/L), Townsend deprivation index (TDI), comorbidities (diabetes, hypertension, angina, heart attack), additional medication use (insulin, hypertension), alcohol consumption (six answer options: never, special occasions only, one to three times a month, once or twice a week, three or four times a week, daily or almost daily; self-reported), smoking status (three answer options: never, previous, current; self-reported), first ten genetic principal components and UKB test centre. Education was recoded as binary depending on whether or not the participants were holding a university/college degree.

3.9 Statistical analysis

The current analyses were conducted using the IBM Statistical Package for Social Science software (SPSS) version 26 (IBM Corp., Chicago, IL, USA). The analyses were restricted to the baseline data for self-reported insomnia and chronotype, the above-mentioned SNPs, statin treatment and covariates from the UK Biobank. For all the variables wherever applicable, the answers “I don’t know” and “Prefer not to answer” were recoded as missing. The genotypes were coded as 0, 1, and 2 (based on the number of risk alleles) and included in regression models as predictor (independent) variable. Insomnia was treated as a dichotomous trait (0 representing “controls” and 1 representing “insomnia cases”) and was tested via binary logistic regression. Chronotype was treated as categorical (1–4 for the respective four answer options) or dichotomous (“definite morning person” set to control and “definite evening person” set to case). The threshold for significance was further adjusted to account for multiple testing, i.e., $p < 0.025$ (0.05/2 parameters).

We first examined if the genetic variants of *HMGCR* and *PCSK9* were associated with lower total cholesterol and LDL-cholesterol levels as well as lower incidence of heart attack in our study population, by using linear regression and logistic regression, respectively. Then,

we compared the prevalence of insomnia and the chronotype in the whole study population by using covariate-adjusted binary and multinomial logistic regression models. Both unadjusted and adjusted analyses were conducted and the results are reported. Analyses were adjusted for age, sex, sleep duration, total cholesterol level, LDL cholesterol level, BMI, smoking, alcohol consumption, heart attack, angina, hypertension, diabetes, education, physical activity, hypertensive medication, insulin use, TDI, first ten genetic principal components and UKB test centre. The covariates were selected for their link with the sleep related outcomes and cardiovascular disease. The association of insomnia with covariates was tested by employing chi-square test for categorical variables (sex, education, alcohol intake, smoking, comorbid conditions, medications and chronotype) and linear regression for continuous variables (age, TDI, physical activity, sleep duration, BMI, total cholesterol and LDL cholesterol). Moreover, a possible interaction effect of statin treatment and the presence of one of the SNPs was investigated using a multiplicative term in the logistic regression models adjusted for the aforementioned covariates.

Lastly, as majority of the statin users (93%) used lipophilic statins, we additionally conducted the analysis for association of statin use with insomnia and chronotype after excluding the hydrophilic statin users ($n = 2739$). Since we observed no significant difference in the findings, the results based on entire sample (i.e., including both hydrophilic and lipophilic statin users) are presented throughout the manuscript.

4. Results

4.1 General prevalence of insomnia

Insomnia, defined as having trouble falling asleep at night or waking up in the middle of the night on a regular basis, was reported by 30.5% of individuals in our study population (mean age \pm SD 57.5 ± 7.9 years). The prevalence was higher in women compared to men (33.8% versus 26.2%, respectively).

4.2 Effect of statin treatment on insomnia

As we described in a previous study [22], statin users differed from controls in a range of health-related parameters (Table 1). In the current study, statin users had 0.95 mmol/L lower LDL cholesterol levels compared to controls [95% CI, 0.94 to 0.96] in the unadjusted analyses and after the adjustment with age and sex, the circulating LDL cholesterol was further decreased to 1.0 mmol/L [95% CI, 0.99 to 1.01]. With respect to sleep, statin users more often reported insomnia symptoms compared to controls, the unadjusted odds ratio (OR) was 1.15 [95% CI, 1.12 to 1.17; $p = 2.89 \times 10^{-41}$] and the adjusted OR was 1.07 [95% CI, 1.03 to 1.11; $p = 1.42 \times 10^{-4}$] (Fig. 2A).

4.3 Effect of HMGCR and PCSK9 genotype on LDL, total plasma cholesterol and coronary artery diseases

We first validated that the *HMGCR* and *PCSK9* gene variants were associated with lower circulating cholesterol as well as a lower incidence of coronary artery diseases in our study population, as shown in other studies [27, 28, 33]. The demographic characteristics of individuals carrying different *HMGCR* and *PCSK9* gene variants are shown in **Supplementary Table 2** and **Supplementary Table 3**.

Each additional *HMGCR* rs17238484-G allele was associated with 0.047 mmol/L lower LDL-cholesterol levels [95% CI, 0.040 to 0.053; $p = 2.50 \times 10^{-45}$] and 0.057 mmol/L lower total cholesterol levels [95% CI, 0.049 to 0.065; $p = 3.16 \times 10^{-40}$] after adjustment with age and sex. The risk for heart attack was lowered with each additional allele by 7.0% [95% CI, 0.89 to 0.98; $p = 0.004$] after adjustment with age, sex, LDL and total cholesterol [27, 33]. Similarly, each additional *HMGCR* rs12916-T allele was associated with 0.056 mmol/L lower LDL-cholesterol levels [95% CI, 0.050 to 0.061; $p = 3.67 \times 10^{-86}$] and 0.069 mmol/L lower total cholesterol levels [95% CI 0.061 to 0.076; $p = 5.4 \times 10^{-78}$] as well as a lower risk for heart attack (OR [95% CI], 0.93 [0.90 to 0.97]; $p = 0.001$).

Similarly, the *PCSK9* rs11591147-T allele was associated with 0.29 mmol/L lower LDL-cholesterol [95% CI, 0.26 to 0.31; $p = 1.30 \times 10^{-158}$] and 0.34 mmol/L lower total cholesterol levels [95% CI, 0.31 to 0.37; $p = 9.92 \times 10^{-133}$]. Moreover, each additional rs11591147-T allele was also associated with 38.0% lower risk for heart attack [95% CI, 0.52 to 0.74; $p = 4.13 \times 10^{-8}$].

4.4 Effect of HMGCR and PCSK9 genotypes on insomnia

After validating our genetic instruments, we analysed the association of rs17238484, rs12916 and rs11591147 genotypes with insomnia symptoms. Interestingly, gene variants in *HMGCR* and *PCSK9* were both associated with insomnia, but in a differential manner (Fig. 2B–D, Table 2). We observed that each additional rs17238484-G allele was associated with 2.0% lower odds for insomnia [95% CI, 0.96 to 0.99; $p = 0.014$] (Fig. 2B). Similarly, the rs12916-T allele was associated with 2.0% lower odds for insomnia [95% CI, 0.96 to 0.99; $p = 0.002$] (Fig. 2C). In contrast, we observed that each additional *PCSK9* rs11591147-T allele was associated with 8.0% higher odds for insomnia [95% CI, 1.02 to 1.14; $p = 0.014$] (Fig. 2D).

4.5 Effect of statin treatment and genetic variants of HMGCR and PCSK9 on chronotype

We did not observe any association of either statin treatment or *HMGCR* gene variants with chronotype (Fig. 3A–C). In contrast, each additional copy of the *PCSK9* rs11591147-T allele was associated with 15.9% greater odds for evening preference [95% CI, 1.06 to 1.30;

Table 1. Baseline characteristics of statin users and non-users in the UK biobank.

Variable	Non-users	Statin users
N	165,372	41,429
<i>Continuous variables, mean (SD)</i>		
Age (years)	56.50 ± 8.00	61.60 ± 5.90
Townsend deprivation index	-1.62 ± 2.89	-1.31 ± 3.08
Physical activity (>10 min moderate activity/day)	3.60 ± 2.32	3.53 ± 2.34
Body mass index (kg/m ²)	27.24 ± 4.73	29.23 ± 4.88
Sleep duration (hours/day)	7.16 ± 1.07	7.24 ± 1.19
Total cholesterol concentration (mmol/L)	5.91 ± 1.07	4.66 ± 0.93
LDL cholesterol (mmol/L)	3.71 ± 0.81	2.77 ± 0.66
<i>Categorical variables, N (%)</i>		
Sex	Male	64,396 (38.9%)
	Female	100,976 (61.1%)
¹ Education	Yes	51,770 (31.3%)
	No	9660 (24.1%)
Alcohol intake	Daily or almost daily	34,767 (21.0%)
	3 to 4 times a week	38,981 (23.6%)
	Once or twice a week	43,611 (26.4%)
	One to three times a month	19,246 (11.6%)
	Special occasions only	17,880 (10.8%)
	Never	10,782 (6.5%)
Smoking	Never	91,729 (55.6%)
	Former	57,114 (34.6%)
	Current	15,994 (9.7%)
Comorbidity	Diabetes	2711 (1.63%)
	Hypertension	42,536 (25.7%)
	Heart attack	964 (0.6%)
	Angina	1457 (0.9%)
Medication use	Antihypertensive	30,542 (18.6%)
	Insulin	709 (0.4%)
Chronotype	Definitely morning	38,930 (26.2%)
	Mild morning	53,933 (36.3%)
	Mild evening	42,734 (28.7%)
	Definitely evening	13,086 (8.8%)
Insomnia symptoms	No	115,831 (70.1%)
	Yes	49,441 (29.9%)

¹Education was dichotomized based on whether the participants hold college/university degree or not.

$p = 0.001$] (Fig. 3D). Moreover, when the analyses were restricted to compare only definitely evening preference with definitely morning preference, *PCSK9* rs11591147-T allele was associated with 17% greater odds for evening preference [95% CI, 1.05 to 1.29; $p = 0.003$] (Table 3).

4.6 Interaction between statin treatment and genetic variation on insomnia

No significant interaction was observed between statin treatment and genetic variation in *HMGCR* or *PCSK9* genetic variants on the risk of having insomnia. Only a nominal significant interaction effect was observed between statin treatment and *HMGCR* rs12916-T (the allele with the greater positive effect on insomnia symptoms) on insomnia symptoms [$P_{int} = 0.025$] (Table 2).

5. Discussion

In the present study, the possible relationship between cholesterol-lowering treatment and sleep disturbances was investigated by using genetic variants in the *HMGCR* and *PCSK9* genes that mimic the effects of cholesterol-lowering drugs (statins and PCSK9 inhibitors), in a large population-based cohort from the UK. Treatment with statins as well as genetic variation mimicking PCSK9 inhibition were found to associate with a greater risk for insomnia, while genetic variants mimicking *HMGCR* inhibition were found to be protective against insomnia. Moreover, the *PCSK9* genetic variant significantly associated with chronotype, while no effect on chronotype was observed in relation to statin use or genetic variation in *HMGCR*.

Table 2. Association of *HMGR* (rs17238484-G and rs12916-T) and *PCSK9* (rs11591147-T) alleles with risk of insomnia.

SNP	Gene	Model	OR	95% CI	<i>p</i>	<i>P_{int}</i>
rs17238484_G	<i>HMGR</i>	Unadjusted	0.98	0.96–0.99	0.008	0.150
		Adjusted	0.98	0.96–0.99	0.014	
rs12916_T	<i>HMGR</i>	Unadjusted	0.98	0.97–0.99	0.004	0.025
		Adjusted	0.98	0.96–0.99	0.002	
rs11591147_T	<i>PCSK9</i>	Unadjusted	1.05	1.00–1.11	0.047	0.163
		Adjusted	1.08	1.02–1.14	0.014	

Tested with logistic regression on self-reported insomnia symptoms. SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; *P_{int}*, *p* for interaction. Analysis adjusted for age, sex, first ten genetic principal components, Townsend deprivation index, UK Biobank test centre, sleep duration, chronotype, body mass index, type 2 diabetes, total plasma cholesterol, LDL cholesterol, statin use, alcohol intake frequency, smoking status, heart attack, angina, hypertension, physical activity, education, antihypertensive medication and insulin medication. The threshold for significance after correction for multiple testing was $p < 0.025$.

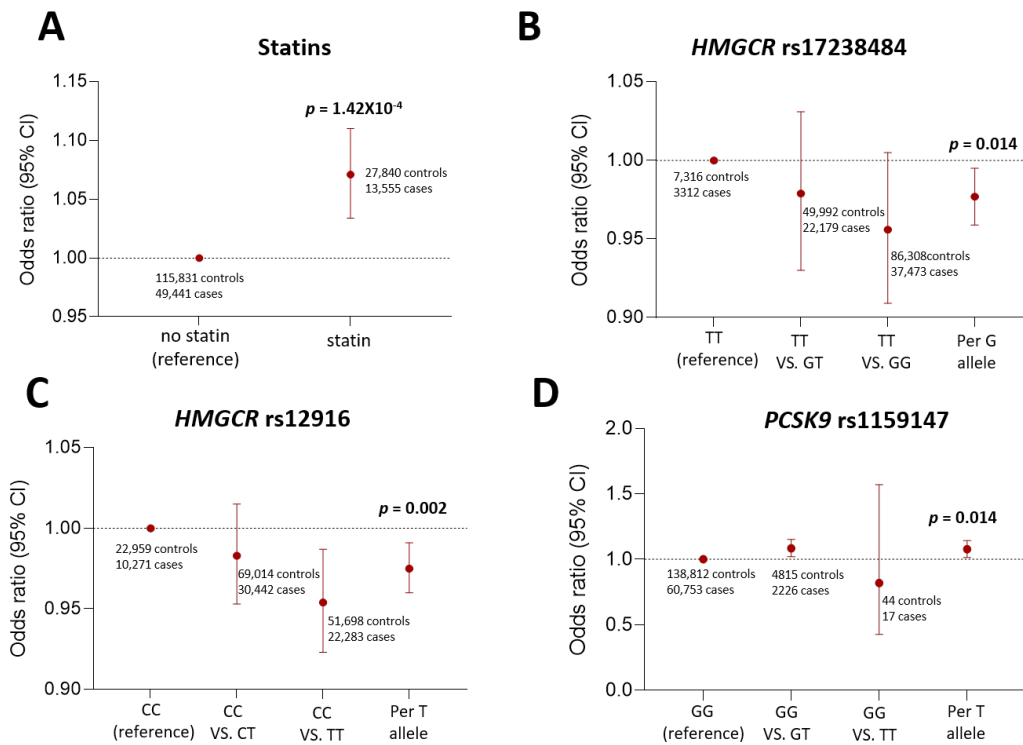


Fig. 2. Association of statin use, *HMGR* rs17238484 and rs12916 as well as *PCSK9* rs1159147 alleles with self-reported insomnia symptoms. Odds ratios of insomnia with (A) statin use and (B–D) different copies of respective gene variants of *HMGR* (B: rs17238484, C: rs12916 and D: rs1159147) were analysed via binary logistic regression, adjusted for age, sex, sleep duration, plasma lipid concentration, BMI, smoking, alcohol consumption, heart attack, angina, hypertension, diabetes, education, physical activity, hypertension medication, insulin medication, Townsend deprivation index, first ten genetic principal components and UK Biobank test centre. The threshold for significance after correction for multiple testing was $p < 0.025$. CI, confidence interval.

We found that the association of statin treatment, as well as its genetic proxies in the *HMGR* gene (the intended drug target), with insomnia are not directionally consistent. *HMGR* genetic variants in the UK population were not associated with a higher risk of insomnia, but instead had protective effects. Statin treatment, on the

other hand, was associated with an increased risk of insomnia, suggesting that these effects are not a consequence of *HMGR* inhibition. First, the effect of *HMGR* on sleep is interesting, as to our knowledge no specific role has so far emerged for *HMGR* in the regulation of sleep behaviour. Further studies investigating the cellular and

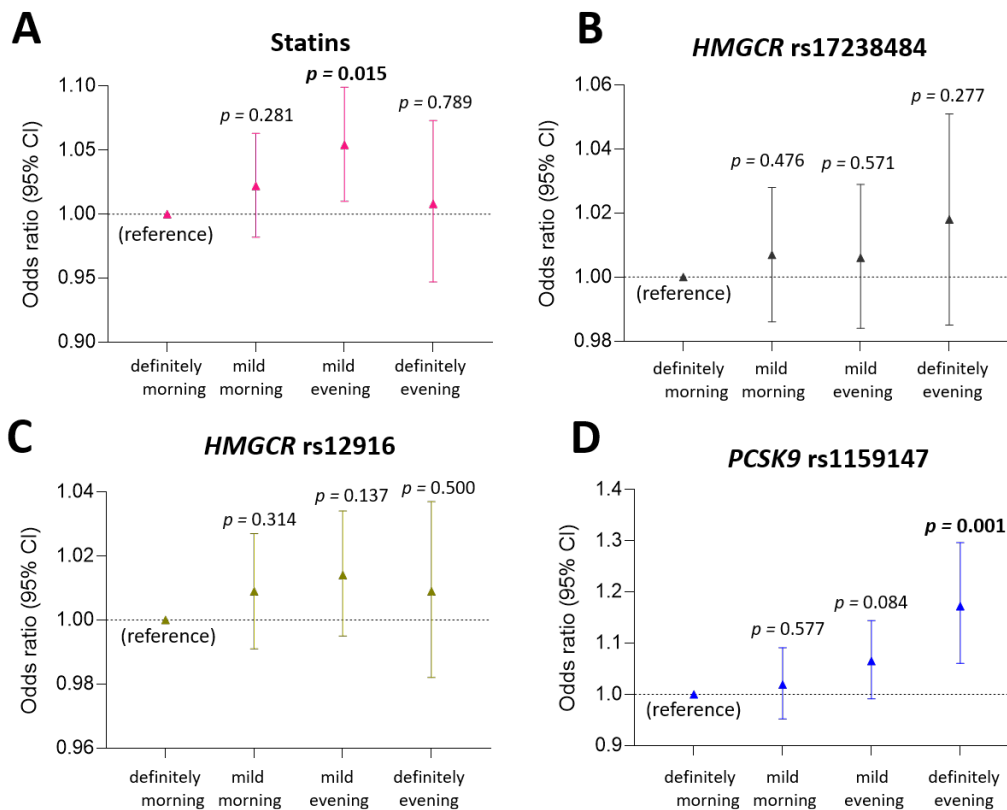


Fig. 3. Association of statin use, HMGCR rs17238484 and rs12916 as well as PCSK9 rs1159147 alleles with self-reported chronotype. Odds ratios of the strength of self-reported morning or evening preference (mild or definite) with (A) statin use and (B–D) different copies of respective gene variants of HMGCR (B: rs17238484, C: rs12916) and PCSK9 (D: rs1159147) were analysed via multinomial logistic regression, adjusted for age, sex, sleep duration, total plasma cholesterol, LDL cholesterol, BMI, smoking, alcohol consumption, heart attack, angina, hypertension, type 2 diabetes, education, physical activity, hypertension medication, insulin medication and Townsend deprivation index, first ten genetic principal components, UK Biobank test centre. The threshold for significance after correction for multiple testing was $p < 0.025$ (significant p values are written in bold letters). CI, confidence interval.

molecular basis of this involvement will be interesting. Secondly, two previously identified off targets of statins might instead be related to the unfavourable effects of statin treatment on sleep, the peroxisome proliferator-activated receptor alpha (PPAR α) (activated by statins) [34] and complex III of the mitochondrial respiratory chain (inhibited by statins) [24]. A connection between mitochondrial respiration and sleep has been described in a mouse model of paradoxical sleep deprivation [35]. Sleep-deprived mice showed reduced mitochondrial respiratory activity, including the activity of complex III in the brain. Whether reduced complex III activity in turn could induce insomnia has to our knowledge not been demonstrated. The role of PPAR α in the regulation of sleep has also been addressed in recent years [36, 37]. It has been concluded that PPAR α activation promotes wakefulness and reduces sleep [38]. Thus, the insomnia-promoting effect of statin treatment might be related to other targets than HMGCR such as PPAR α . We also found that rs1159147 within the PCSK9 locus, a genetic variant that acted as a proxy for a com-

paratively new cholesterol lowering treatment (PCSK9 inhibitors), was significantly associated with chronotype as well as increased risk of insomnia, suggesting a role of PCSK9 in the regulation of circadian rhythm. While nothing specific is known about a function of PCSK9 in regulating sleep behaviour, a sleep-SNP relationship has recently been demonstrated for PCSK9 in a large genome-wide association study targeting the link between long and short sleep duration and the blood lipid profile [39]. Thus, our study adds interesting new data that encourages further analyses into the underlying mechanisms of sleep regulation by PCSK9.

While HMGCR and PCSK9 seem to play a similar role in the development of type-2 diabetes [27, 28], the current results indicate that they act differently on sleep behaviour. This seems to indicate that cholesterol lowering is not the mechanism underlying the development of insomnia symptoms. It is possible that different roles of the two proteins in different tissues (e.g., liver versus brain) are responsible for their opposing effect on sleep. Following this hy-

Table 3. Association of *HMGC*R (rs17238484-G and rs12916-T) and *PCSK*9 (rs11591147-T) alleles with chronotype.

SNP	Gene	Model	OR	95% CI	<i>p</i>
rs17238484_G	<i>HMGC</i> R	Unadjusted	1.02	0.99–1.06	0.13
		Adjusted	1.02	0.99–1.05	0.24
rs12916_T	<i>HMGC</i> R	Unadjusted	1.02	0.99–1.04	0.20
		Adjusted	1.01	0.98–1.04	0.39
rs11591147_T	<i>PCSK</i> 9	Unadjusted	1.15	1.04–1.26	0.004
		Adjusted	1.17	1.05–1.29	0.003

Tested with logistic regression on extreme chronotypes (definite morning vs definite evening type). SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval. Analysis adjusted for age, sex, first ten genetic principal components, Townsend deprivation index, UK Biobank test centre, sleep duration, chronotype, body mass index, type 2 diabetes, total plasma cholesterol, LDL cholesterol, statin use, alcohol intake frequency, smoking status, heart attack, angina, hypertension, physical activity, education, antihypertensive medication and insulin medication. Note: increased OR indicates greater evening preference. The threshold for significance after correction for multiple testing was $p < 0.025$.

pothesis, individual statins with different tissue disposition properties may exert different effects on sleep behaviour, contributing to different study outcomes. The majority of statin users in our study had prescriptions for simvastatin, a lipophilic statin with high tissue penetration (and brain permeability) [40, 41], while hydrophilic statins were only used by a minor fraction (1.3%) of our study cohort.

There are several strengths and limitations of this study that need to be acknowledged. The biggest strengths are that the study used an extraordinarily large sample size and included both genetic and phenotypic data. Moreover, the comprehensive dataset allowed for the adjustment for several potential confounding factors to minimize bias. Still, some limitations should also be considered. First data on sleep was self-reported, and there are indications that some self-reported side effects of statins might underlie a placebo effect, meaning that statin users might report negative side effects to a greater extent than control persons due to higher awareness or negative expectations [15, 42]. Thus, we cannot rule out that a placebo effect also played a role in this study. Second, there is no sufficient data on dose and duration of statin treatment available in the UK Biobank, and most participants on statin treatment have been using simvastatin, which limits the conclusions that can be drawn with respect to statin pharmacology. Third, due to unavailability of data on use of PCSK9 inhibitors, it was impossible to compare the effect of drug use versus genetic variant on the studied sleep parameters as it was possible for statins. Finally, we restricted the analysis to the European ancestry to reduce specific bias in our genetic analyses, such as population stratification, limiting the translatability to other ancestries.

6. Conclusions

In summary, our study revealed interesting new results on the effect of cholesterol-lowering therapy on sleep behaviour, which contributes to our understanding of the multifaceted, seemingly controversial outcomes of statin effects in literature. Further investigations may address for instance more or different sleep parameters, the effect of statin type, and effects in non-European study cohorts. Our results also encourage further mechanistic studies to decipher the mechanism behind the differential effects of statins, *HMGC*R and *PCSK*9 and the potential role of *HMGC*R and *PCSK*9 in sleep and other circadian rhythms in cell or animal models.

7. Author contributions

AMA, XT, DC, MJW, CB and HBS conceived and designed the study. AMA, GR, DC, XT and MHA performed the statistical analysis. AMA, GR, LEC, CB and HBS wrote the manuscript and interpreted the data; All authors revised the paper.

8. Ethics approval and consent to participate

All participants in the UK Biobank study have given informed consent for data collection, data storage and subsequent data analysis. Ethical permission for our study was given by the responsible local authorities, *Regionala Etikprövningsnämnden* (now *Etikprövningsmyndigheten*, Swedish Ethical Review Authority, EPN), Uppsala, under registration number (*Diarienummer*, Dnr) 2017/198.

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11. Conflict of interest

The authors declare no conflict of interest.

12. References

- [1] Cholesterol Treatment Trialists' (CTT) Collaboration, Baigent C, Blackwell L, Emberson J, Holland LE, Reith C, *et al.* Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. *The Lancet*. 2010; 376: 1670–1681.
- [2] Istvan ES, Deisenhofer J. Structural mechanism for statin inhibition of HMG-CoA reductase. *Science*. 2001; 292: 1160–1164.
- [3] O'Regan C, Wu P, Arora P, Perri D, Mills EJ. Statin therapy in stroke prevention: a meta-analysis involving 121,000 patients. *The American Journal of Medicine*. 2008; 121: 24–33.
- [4] Pencina MJ, Navar-Boggan AM, D'Agostino RB, Williams K, Neely B, Sniderman AD, *et al.* Application of new cholesterol guidelines to a population-based sample. *The New England Journal of Medicine*. 2014; 370: 1422–1431.
- [5] Koren MJ, Giugliano RP, Raal FJ, Sullivan D, Bolognese M, Langslet G, *et al.* Efficacy and safety of longer-term administration of evolocumab (AMG 145) in patients with hypercholesterolemia: 52-week results from the Open-Label Study of Long-Term Evaluation against LDL-C (OSLER) randomized trial. *Circulation*. 2014; 129: 234–243.
- [6] Robinson JG, Farnier M, Krempf M, Bergeron J, Luc G, Averna M, *et al.* Efficacy and safety of alirocumab in reducing lipids and cardiovascular events. *The New England Journal of Medicine*. 2015; 372: 1489–1499.
- [7] Ajufo E, Rader DJ. Recent advances in the pharmacological management of hypercholesterolaemia. *The Lancet. Diabetes and Endocrinology*. 2016; 4: 436–446.
- [8] Awan Z, Seidah NG, MacFadyen JG, Benjannet S, Chasman DI, Ridker PM, *et al.* Rosuvastatin, proprotein convertase subtilisin/kexin type 9 concentrations, and LDL cholesterol response: the JUPITER trial. *Clinical Chemistry*. 2012; 58: 183–189.
- [9] Takada M, Fujimoto M, Yamazaki K, Takamoto M, Hosomi K. Association of statin use with sleep disturbances: data mining of a spontaneous reporting database and a prescription database. *Drug Safety*. 2014; 37: 421–431.
- [10] Szmyd B, Rogut M, Białasiewicz P, Gabrylska A. The impact of glucocorticoids and statins on sleep quality. *Sleep Medicine Reviews*. 2021; 55: 101380.
- [11] Broncel M, Gorzelak-Pabiś P, Sahebkar A, Serejko K, Ursolnik S, Rysz J, *et al.* Sleep changes following statin therapy: a systematic review and meta-analysis of randomized placebo-controlled polysomnographic trials. *Archives of Medical Science*. 2015; 11: 915–926.
- [12] Morin CM, Drake CL, Harvey AG, Krystal AD, Manber R, Riemann D, *et al.* Insomnia disorder. *Nature Reviews. Disease Primers*. 2015; 1: 15026.
- [13] Caap-Ahlgren M, Dehlin O. Insomnia and depressive symptoms in patients with Parkinson's disease. Relationship to health-related quality of life. an interview study of patients living at home. *Archives of Gerontology and Geriatrics*. 2001; 32: 23–33.
- [14] Daley M, Morin CM, LeBlanc M, Grégoire JP, Savard J. The economic burden of insomnia: direct and indirect costs for individuals with insomnia syndrome, insomnia symptoms, and good sleepers. *Sleep*. 2009; 32: 55–64.
- [15] Ward NC, Watts GF, Eckel RH. Statin Toxicity. *Circulation Research*. 2019; 124: 328–350.
- [16] Barth JD, Kruisbrink OA, Van Dijk AL. Inhibitors of hydroxymethylglutaryl coenzyme a reductase for treating hypercholesterolaemia. *British Medical Journal*. 1990; 301: 669.
- [17] Grundy SM. HMG-CoA reductase inhibitors for treatment of hypercholesterolemia. *The New England Journal of Medicine*. 1988; 319: 24–33.
- [18] Eckernäs SA, Roos BE, Kvidal P, Eriksson LO, Block GA, Neufus RP, *et al.* The effects of simvastatin and pravastatin on objective and subjective measures of nocturnal sleep: a comparison of two structurally different HMG CoA reductase inhibitors in patients with primary moderate hypercholesterolaemia. *British Journal of Clinical Pharmacology*. 1993; 35: 284–289.
- [19] Sinzinger H, Mayr F, Schmid P, Granegger S, O'Grady J, Peskar BA. Sleep disturbance and appetite loss after lovastatin. *The Lancet*. 1994; 343: 973.
- [20] Black DM, Lamkin G, Olivera EH, Laskarzewski PM, Stein EA. Sleep disturbance and HMG CoA reductase inhibitors. *Journal of the American Medical Association*. 1990; 264: 1105.
- [21] Sierra S, Ramos MC, Molina P, Esteo C, Vázquez JA, Burgos JS. Statins as neuroprotectants: a comparative in vitro study of lipophilicity, blood-brain-barrier penetration, lowering of brain cholesterol, and decrease of neuron cell death. *Journal of Alzheimer's disease*. 2011; 23: 307–318.
- [22] Alsehli AM, Olivo G, Clemensson LE, Williams MJ, Schiöth HB. The Cognitive Effects of Statins are Modified by Age. *Scientific Reports*. 2020; 10: 6187.
- [23] Power MC, Weuve J, Sharrett AR, Blacker D, Gottesman RF. Statins, cognition, and dementia—systematic review and methodological commentary. *Nature Reviews. Neurology*. 2015; 11: 220–229.
- [24] Schirris TJ, Renkema GH, Ritschel T, Voermans NC, Bilos A, van Engelen BG, *et al.* Statin-Induced Myopathy is Associated with Mitochondrial Complex III Inhibition. *Cell Metabolism*. 2015; 22: 399–407.
- [25] Niedzielski M, Broncel M, Gorzelak-Pabiś P, Woźniak E. New possible pharmacological targets for statins and ezetimibe. *Biomedicine and Pharmacotherapy*. 2020; 129: 110388.
- [26] Murphy C, Deplazes E, Cranfield CG, Garcia A. The Role of Structure and Biophysical Properties in the Pleiotropic Effects of Statins. *International Journal of Molecular Sciences*. 2020; 21: 8745.
- [27] Nelson CP, Lai FY, Nath M, Ye S, Webb TR, Schunkert H, *et al.* Genetic Assessment of Potential Long-Term on-Target Side Effects of PCSK9 (Proprotein Convertase Subtilisin/Kexin Type 9) Inhibitors. *Circulation. Genomic and Precision Medicine*. 2019; 12: e002196.
- [28] Swerdlow DI, Preiss D, Kuchenbaecker KB, Holmes MV, Engmann JEL, Shah T, *et al.* HMG-coenzyme a reductase inhibition, type 2 diabetes, and bodyweight: evidence from genetic analysis and randomised trials. *The Lancet*. 2015; 385: 351–361.
- [29] Bycroft C, Freeman C, Petkova D, Band G, Elliott LT, Sharp K, *et al.* The UK Biobank resource with deep phenotyping and genomic data. *Nature*. 2018; 562: 203–209.
- [30] Benn M, Nordestgaard BG, Grande P, Schnohr P, Tybjaerg-Hansen A. PCSK9 R46L, low-density lipoprotein cholesterol levels, and risk of ischemic heart disease: 3 independent studies and meta-analyses. *Journal of the American College of Cardiology*. 2010; 55: 2833–2842.
- [31] Jansen PR, Watanabe K, Stringer S, Skene N, Bryois J, Hammerschlag AR, *et al.* Genome-wide analysis of insomnia in 1,331,010 individuals identifies new risk loci and functional pathways. *Nature Genetics*. 2019; 51: 394–403.
- [32] Lane JM, Jones SE, Dashti HS, Wood AR, Aragam KG, van Hees VT, *et al.* Biological and clinical insights from genetics of insomnia symptoms. *Nature Genetics*. 2019; 51: 387–393.
- [33] Ference BA, Robinson JG, Brook RD, Catapano AL, Chapman MJ, Neff DR, *et al.* Variation in PCSK9 and HMGCR and Risk of Cardiovascular Disease and Diabetes. *The New England Journal of Medicine*. 2016; 375: 2144–2153.
- [34] Roy A, Jana M, Kundu M, Corbett GT, Rangaswamy SB, Mishra RK, *et al.* HMG-CoA Reductase Inhibitors Bind to PPAR α to Upregulate Neurotrophin Expression in the Brain and Improve Memory in Mice. *Cell Metabolism*. 2015; 22: 253–265.
- [35] Andrezza AC, Andersen ML, Alvarenga TA, de-Oliveira MR, Armani F, Ruiz FS, *et al.* Impairment of the mitochondrial electron transport chain due to sleep deprivation in mice. *Journal of Psychiatric Research*. 2010; 44: 775–780.
- [36] Murillo-Rodríguez E, Guzmán K, Arankowsky-Sandoval G, Salas-Crisóstomo M, Jiménez-Moreno R, Arias-Carrión O.

Evidence that activation of nuclear peroxisome proliferator-activated receptor alpha (PPAR α) modulates sleep homeostasis in rats. *Brain Research Bulletin*. 2016; 127: 156–163.

- [37] Mijangos-Moreno S, Poot-Aké A, Guzmán K, Arankowsky-Sandoval G, Arias-Carrión O, Zaldívar-Rae J, *et al.* Sleep and neurochemical modulation by the nuclear peroxisome proliferator-activated receptor α (PPAR- α) in rat. *Neuroscience Research*. 2016; 105: 65–69.
- [38] Murillo-Rodríguez E. The Role of Nuclear Receptor PPAR α in the Sleep-wake Cycle Modulation. a Tentative Approach for Treatment of Sleep Disorders. *Current Drug Delivery*. 2017; 14: 473–482.
- [39] Noordam R, Bos MM, Wang H, Winkler TW, Bentley AR, Kilpeläinen TO, *et al.* Multi-ancestry sleep-by-SNP interaction analysis in 126,926 individuals reveals lipid loci stratified by sleep duration. *Nature Communications*. 2019; 10: 5121.
- [40] Botti RE, Triscari J, Pan HY, Zayat J. Concentrations of pravastatin and lovastatin in cerebrospinal fluid in healthy subjects. *Clinical Neuropharmacology*. 1991; 14: 256–261.
- [41] Vuletic S, Riekse RG, Marcovina SM, Peskind ER, Hazzard WR, Albers JJ. Statins of different brain penetrability differentially affect CSF PLTP activity. *Dementia and Geriatric Cognitive Disorders*. 2006; 22: 392–398.
- [42] M.P.A. Report, Statins: Updates to Product Safety Information. November 2009. Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/852414/Statins_update_to_product_safety_information.pdf (Access: 15 July 2021).

https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/852414/Statins_update_to_product_safety_information.pdf (Access: 15 July 2021).

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Abbreviations: CVD, cardiovascular disease; HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; LDL, low-density lipoprotein; PCSK9, proprotein convertase subtilisin/kexin type 9; angiotensin II; TDI, townsend deprivation index.

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