Genetic insights into statin-associated diabetes risk

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Word count: 2,353 (abstract 196)

Figures: 1

Tables: 1

Abstract

Purpose of review: Meta-analyses of major statin trials have suggested that statin therapy modestly increases the risk of developing diabetes. However, the quality of the data on which these findings are based is not without weaknesses and it has also been unclear whether this effect, if true, is an on- or off-target effect of statins.

Recent findings: In a major Mendelian randomisation study of variants in the HMGCR gene, which encodes the protein through which statins exert their effect, two polymorphisms associated with lower LDL-cholesterol were also associated with higher weight, higher waist circumference, higher glucose and higher diabetes risk. These findings correspond with findings from the statin trials. In addition, new observational studies using a genetic risk score for LDL-cholesterol suggest that other pathways linked to LDL-cholesterol metabolism may also affect diabetes risk.

Summary: Genetic studies indicate that the observed effect of statins on diabetes risk in trials is highly likely to be a true on-target effect. While other recent studies have suggested that genetically determined lower LDL-cholesterol may be linked to diabetes risk, further data from both genetic studies and clinical trials of other LDL-cholesterol lowering agents are needed to confirm or refute this.

Keywords: statin, diabetes, mendelian randomisation, randomised trial

Introduction

The ability of statins to reduce risk of cardiovascular events in primary and secondary prevention populations has been confirmed in a variety of randomized trials[1]. As a result, statin therapy has become commonplace and the availability of high quality data has allowed statins to become one of the best studied medicines. These trials have also confirmed the safety of statin therapy based on the lack of any adverse effect on important events such as the development of cancer and non-cardiovascular death[2]. Apart from the very infrequent occurrence of myositis or rhabdomyolysis, typically on high dose simvastatin and apparently linked to other risk factors like the use of amiodarone or genetic variants in *SLCO1B1*, plus occasional increases in transaminases typically on high dose atorvastatin, the only other concern that has arisen in twenty years of intensive research is that of a potential increase in the risk of developing type 2 diabetes[3,4] on a statin. It is important to immediately stress, however, that any such risk is very heavily outweighed by the cardiovascular benefit obtained from statins.

The possibility that statins might increase diabetes risk was suggested in an initial pooled analysis of 90,000 patients from 13 statin trials[3]. This finding was far from statistical reproach, despite the vast numbers of participants available, suggesting the likelihood of a modest effect but not dispelling the possibility of unmeasured bias. Additional weaknesses in this analysis were the lack of individual participant data and the necessity to use a variety of definitions for diabetes depending on what was available for each trial. A subsequent pooling of five dose-comparison statin trials with 30,000 participants yielded a similar result[4], adding credence to the statin-diabetes link, though with the same caveats as before. Various plausible suggestions were made to suggest the potential of bias, even in the context of randomized trials[5]. In addition, a plethora of observational studies, all confounded to a greater or lesser extent by virtue of their design, investigated the same topic and

produced a wide variety of results[6,7]. There was therefore a need for high quality, unbiased data, even in the context of the wealth of available statin trials.

Consideration of how to conduct further meaningful research to confirm or refute this finding offered few viable options. Various biomarker studies in the past had failed to conclusively address this issue[8] and alternative approaches such as euglycaemic insulin clamp studies, while academically interesting, had been equally unhelpful based on their high intra-individual variability. However, detailed knowledge of the mechanism of statins' effect, namely inhibition of hydroxymethylglutaryl-CoA reductase (HMGCR), and evidence from robust GWAS studies of the genetic determinants of LDL-cholesterol that included variants at the *HMGCR* locus[9], indicated that this may represent an attractive approach with which to study this issue. In addition, the biochemical nature of diabetes presented the attractive opportunity to study not only the binary outcome of diabetes but also related continuous measures such as weight, glucose, and insulin levels. On that basis, an approach which combined randomised trial data and genetic polymorphism data allowed two complementary approaches to be employed to investigate this effect.

HMGCR and diabetes risk: Mendelian Randomisation

Genetic studies in populations offer an ideal setting in which to investigate on-target drug effects. The majority of drug targets are proteins and, as such, are encoded by one (or occasionally more than one) gene. HMGCR is one such protein drug target, encoded by the *HMGCR* gene. Under the Mendelian randomisation principle, common variants in genes encoding a drug target can be used as reliable proxies for pharmacological modulation of that target (see **Figure 1**). This assertion relies on the random allocation of alleles for a given variant at the time of conception, and Mendel's law of independent assortment of alleles. On this basis, observed phenotypic associations between a genetic variant involved in determining the amount, structure or function of a protein drug target

reflect the likely consequences of perturbing that target with a drug molecule[10]. This approach was used to investigate whether statin-induced diabetes is an on-target effect of the drugs (i.e. mediated through inhibition of HMGCR), and, through what biological pathways increased diabetes risk was incurred[11]. In a large dataset including up to 223,463 individuals from 43 studies, the phenotypic associations of two single nucleotide polymorphisms (SNPs) at the *HMGCR* locus (rs12916 and rs17238484) were examined. Both SNPs were strongly associated with lower circulating total, LDL-, and non-HDL-cholesterol although the magnitude of these associations was small: each additional copy of the effect allele at each SNP was associated with 0.08mmol/L and 0.06mmol/L lower LDL-cholesterol respectively (Table 1). The LDL-cholesterol-lowering alleles of the same variants were also associated with higher type 2 diabetes risk (rs12916 odds ratio 1.06 [95% CI 1.03–1.09]; rs17238484 odds ratio 1.02 [1.00-1.05]). Furthermore, the same alleles were associated with higher plasma glucose and insulin, higher body weight, BMI and waist and hip circumferences. The genetic associations were compared with the effects of statin treatment on similar traits in an updated meta-analysis of trial data including information on up to 129,170 individuals from 20 trials. The trial data confirmed statin treatment increased type 2 diabetes risk, and also demonstrated a novel effect of statin treatment on modestly higher body weight (0·24 kg, 95% CI 0·10–0·38) over mean follow-up of 4.2 years. The strong directional concordance between the genetic and trial findings suggested that the higher diabetes risk caused by statins is at least partly an on-target effect, and its underlying mechanism likely involves weight gain. It is possible, however, that other biochemical mechanisms are at play in the relationship between HMGCR inhibition and diabetes risk, and that statin drug molecules could have additional off-target effects that contribute to this risk. This large-scale evaluation of both genetic and clinical trial data has provided strong evidence for a mechanistic link between lipid metabolism and glycaemic control, and offers a framework for investigation of the roles of other biochemical pathway in these two areas.

LDL-cholesterol and diabetes risk

An immediate question regarding the link between HMGCR inhibition and diabetes risk is whether it is limited to this metabolic pathway or whether other LDL metabolic pathways, or indeed other pathways affecting the metabolism of other lipoproteins, have a similar effect. To that end, various studies have recently been published using different approaches but all based on genetic principles. In a recent analysis of the world-leading Dutch database for familial hypercholesterolaemia, a condition now thought to affect one in 200-300 people and characterised by substantial elevations in LDL-cholesterol due to a mutation in one of three genes (*LDLR*, *PCSK9* or *APOB*), it was noted that diabetes risk in those with genetically confirmed FH was 38% lower (51% lower in adjusted analysis) than in unaffected relatives[12] at the time of genetic screening. Notably, risk was lower across all three gene mutations and not limited to *LDLR*, the mutation most plausibly linked to the effect of statins. These results are consistent with the statin-diabetes findings though, again, not beyond reproach given the cross-sectional nature of the study. A further intriguing question is whether the approximately 1kg/m^2 lower BMI noted in FH mutation carrying patients compared to their relatives without mutations was causally related to LDL-cholesterol metabolism (as was suggested in recent statin trial work where statin recipients gained a small amount of weight) or whether it was simply a confounder (as suggested by the difference in smoking prevalence) which partly explains the difference in reported diabetes prevalence. The authors of this report hypothesised that expression of the LDL-receptor in pancreatic tissue and LDL receptor-mediated transmembrane cholesterol transport may link these findings.

Other groups have used a different type of analysis, namely a LDL-cholesterol gene score as the instrumental variable of choice, to further examine the relationship between circulating LDL-cholesterol and diabetes risk. In an analysis of the Malmo Diet and Cancer Study of 27,254 individuals without diabetes followed for 15 years, a one standard deviation reduction in a weighted LDL-cholesterol gene risk score was associated with a doubling in the risk of diabetes and, as

expected, a lower risk of cardiovascular disease[13]. A similar approach was taken by Fall and colleagues in their analysis of major datasets (DIAGRAM, MAGIC, GENESIS) and, again, genetically determined lower LDL-cholesterol was found to be associated with higher diabetes risk[14]. The authors of this report did, however, urge caution in concluding that their data showed a definite causal relationship between LDL-cholesterol and diabetes risk.

The emergence of proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, injectable monoclonal antibodies which typically lower circulating LDL-cholesterol by 50-60%, represents an important opportunity to further study the relationship between LDL-cholesterol metabolism and diabetes risk using complementary trial and genetic approaches, and such work is already underway. Early examples of genetic studies include a recent analysis of the p.R46L variant in *PCSK9* in 5,972 individuals from two French studies[15]. The LDL-cholesterol of carriers of the variant was 0.4mmol/L lower than non-carriers. No differences were noted between carriers and non-carriers in terms of weight, plasma glucose or diabetes risk. An additional drug of interest with an unrelated mechanism of LDL-cholesterol lowering action is ezetimibe, whose target is encoded by the Niemann-Pick C1-Like 1 (*NPC1L1*) gene. Publication of data regarding new-onset diabetes in the IMPROVE-IT ezetimibe trial are now awaited, though the modest effect of the drug on LDL-cholesterol may be insufficient for the available trial data to comprehensively clarify any relationship with new-onset diabetes[16], highlighting the role that analyses of genetic polymorphisms can play when trial data are insufficient.

Metabolism of other lipoproteins and potential impacts on diabetes risk

While the relationship of statins and LDL-cholesterol with diabetes risk has received the greatest attention based on the rich availability of both trial and genetic data, the use of appropriately selected genetic variants to study the possible on-target effects of other lipid-modifying approaches promises to provide information regarding approaches to modify other lipoproteins. Indeed, it is

likely to be a method to not only identify promising strategies for targeting with medicines, but also one to study some potential on-target adverse effects of putative or new therapies. One known side-effect of niacin, an agent which increases HDL-c and lowers LDL-cholesterol and triglycerides, is that it moderately increases blood glucose and the risk of developing diabetes[17,18]. How the lipid changes interact with diabetes risk and whether this is an on- or off-target effect is unclear but the use of a suitable genetic target may elucidate this. Mendelian randomisation studies have shown that loss-of-function apolipoprotein C3 mutations lead to lower levels of triglycerides and reduced risk of cardiovascular disease[19] highlighting the potential of suitable interventions such as an RNA antisense injectable molecule which is currently under investigation. The potential of such an intervention to impact favourably on diabetes risk is suggested by data from a study of 95 health Asian men in which two apolipoprotein C3 SNPs associated with hypertriglyceridaemia also demonstrated a marked reduction in plasma triglyceride clearance, far higher prevalence of non-alcoholic fatty liver disease and lower insulin-sensitivity index[20]; these findings were confirmed in a validation study of 163 healthy non-Asian men. A final example of where such an approach is being used is to investigate cholesteryl ester transfer protein (CETP). Although three major trials of different CETP inhibitors have failed to demonstrate cardiovascular benefit, torcetrapib, an agent with off-target effects which raised blood pressure[21], was nonetheless shown to improve glycaemia in those with and without diabetes[22]. Whether this finding is on- or off-target, and whether it is applicable to other CETP inhibitors is under investigation using the combined approach of genetic and trial data.

Conclusion

Evidence is mounting from both clinical trials and observational studies that regulation of plasma lipids and glycaemic control are more closely linked than previously supposed. A number of lipid-modifying therapies have shown concomitant effects on diabetes risk, despite operating through distinct biochemical mechanisms. An important challenge presents itself: the burden of diabetes on

the global population is growing and carries substantial increased risk of cardiovascular sequelae, but novel lipid-modifying agents are needed to attenuate the CVD risk. Whilst the adverse effects on dysglycaemia observed to-date have been modest compared with the benefits offered by the drugs for CVD prevention, the widening use of lipid-modifying drugs requires greater consideration of their potential adverse effects at the population level. In this regard, greater understanding is required of the evidently complex relationship between lipid metabolism and glycaemic control. Genetic studies, clinical trials and other strategies have a great deal to contribute to this effort, and the findings will have important implications for individual and population health.

3-5 key points

- Data from 20 major statin trials have suggested that statins raise the risk of developing diabetes in a dose-dependent fashion
- However, these trials were designed to study the effect of statins on cardiovascular events, not diabetes risk, with the result that further conclusive evidence would be valuable
- In a major Mendelian randomisation study of variants in the HMGCR gene, two polymorphisms associated with lower LDL-cholesterol were also associated with higher diabetes risk and with traits related to diabetes risk; recent studies of genetic risk scores suggest that other LDL-cholesterol pathways may also impact on diabetes risk
- The modest diabetogenic effect of statins appears to be true and an on-target effect of HMGCR inhibition

Acknowledgements: none

Financial support and sponsorship: none

Conflicts of interest: DP attended advisory board meetings for Sanofi Aventis regarding PCSK9 inhibitors on three occasions in prior employment and is/has been involved in clinical trial programs of PCSK9 inhibitors for Amgen and Pfizer. DIS consults for Pfizer on research unrelated to this work.

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This study presented data from 20 statin trials and showed that moderate statin therapy increases diabetes risk by about 10% compared to control and that intensive statin further increases the risk by about another 10%. Statins also lead to a limited 0.25kg in body weight. In the same paper, data for variants in the HMGCR gene were examined across 43 studies with up to 220,000 individuals. As with statin therapy, those with lower LDL-cholesterol due to polymorphisms in the HMGCR gene were also at higher diabetes risk and had higher weight.

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In this analysis of the Netherland's world-leading familial hypercholesterolaemia(FH) program, the prevalence of diabetes at the time of genetic screening for the condition was examined. It was found that those with FH were at about half the diabetes risk of those without FH and that those with receptor negative FH were at lower diabetes risk than those with receptor defective FH.

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In this analysis of 140 lipid-associated genetic variants and diabetes risk based on major consortia such as DIAGRAM, MAGIC and GENESIS, the investigators sought to study the relationships between genetically determined levels of LDL-cholesterol, HDL-cholesterol and triglycerides. There was weak evidence for a possible causal relationship between lower HDL-cholesterol adnd higher diabetes risk, and stronger evidence for lower LDL-cholesterol. However, based on various sensitivity analyses, the authors urged caution in concluding that their data provided definitive evidence regarding lipids and diabetes risk.

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In this study, PCSK9 p.R46L was genotyped in 4630 French participants from the DESIR prospective study and 1342 French participants with type 2 diabetes. While the variant was associated with 0.39mmol/L lower LDL-cholesterol, markers of glucose homeostasis (fasting glucose, insulin, HbA1c) and type 2 diabetes risk were not affected.

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Tables

Table 1. Associations of HMGCR polymorphisms with LDL-cholesterol and traits related to diabetes risk

	Per additional rs17238484 allele	**Per additional rs12916 allele**
LDL-cholesterol	↓ 0.06 mmol/L (0.05-0.07)	↓ 0.08mmol/L (0.07-0.09)
Weight	↑ 0.30 kg (0.18-0.43)	↑ 0.20kg (0.04-0.36)
Waist circumference	↑ 0.32 cm (0.16-0.47)	↑ 0.30cm (0.10-0.50)
Glucose	↑ 0.23% (0.02-0.44)	0.13 (-0.14-0.39)
Insulin	↑ 1.62% (0.53-2.72)	0.66% (-0.63 to 1.97)
T2DM	1.02 (1.00-1.05)	↑ 1.06 (1.03-1.09)

Figures.

Figure 1. Comparison of statin trial and HMGCR Mendelian randomisation methodologies

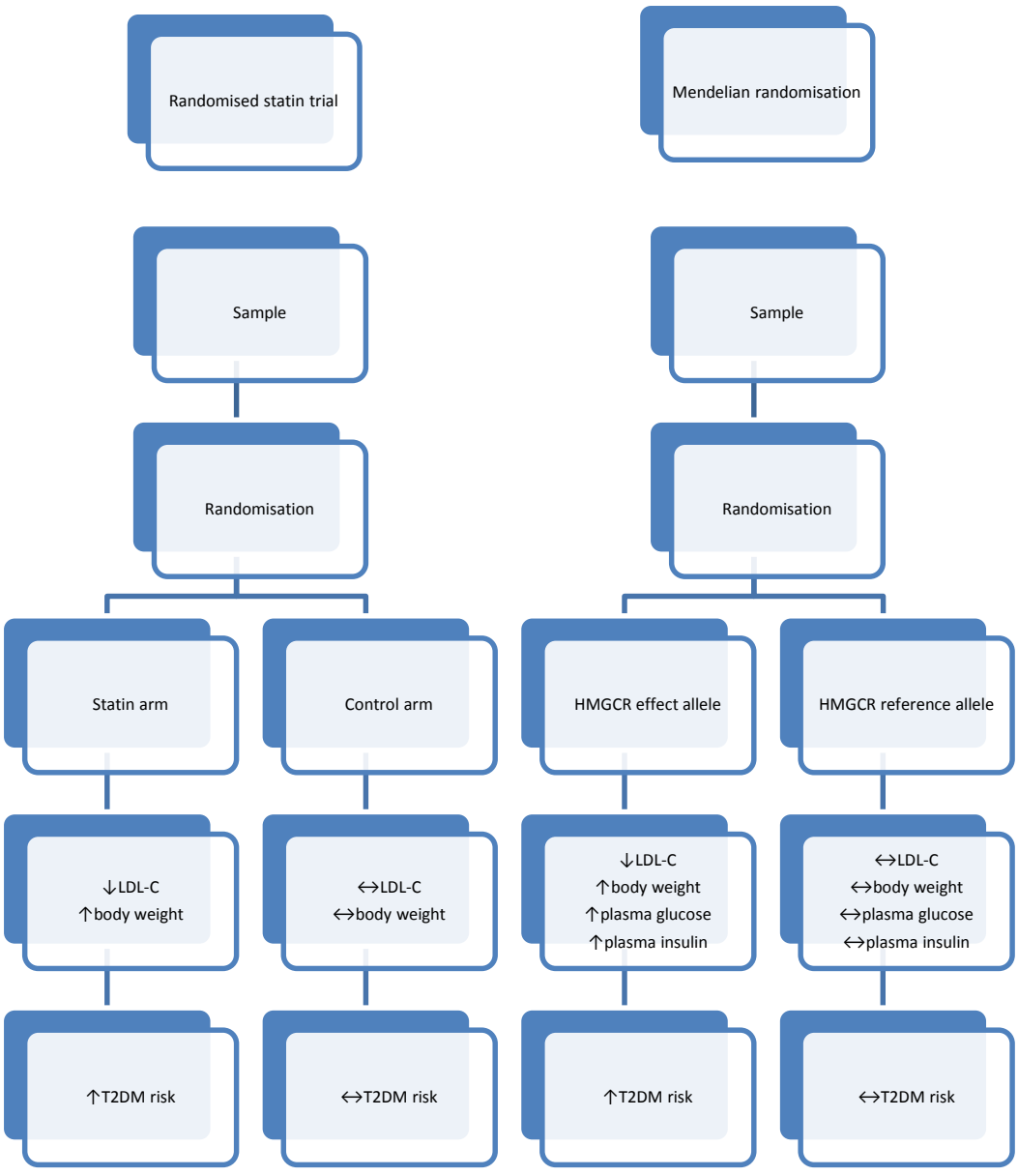

Figure 1

Figures.

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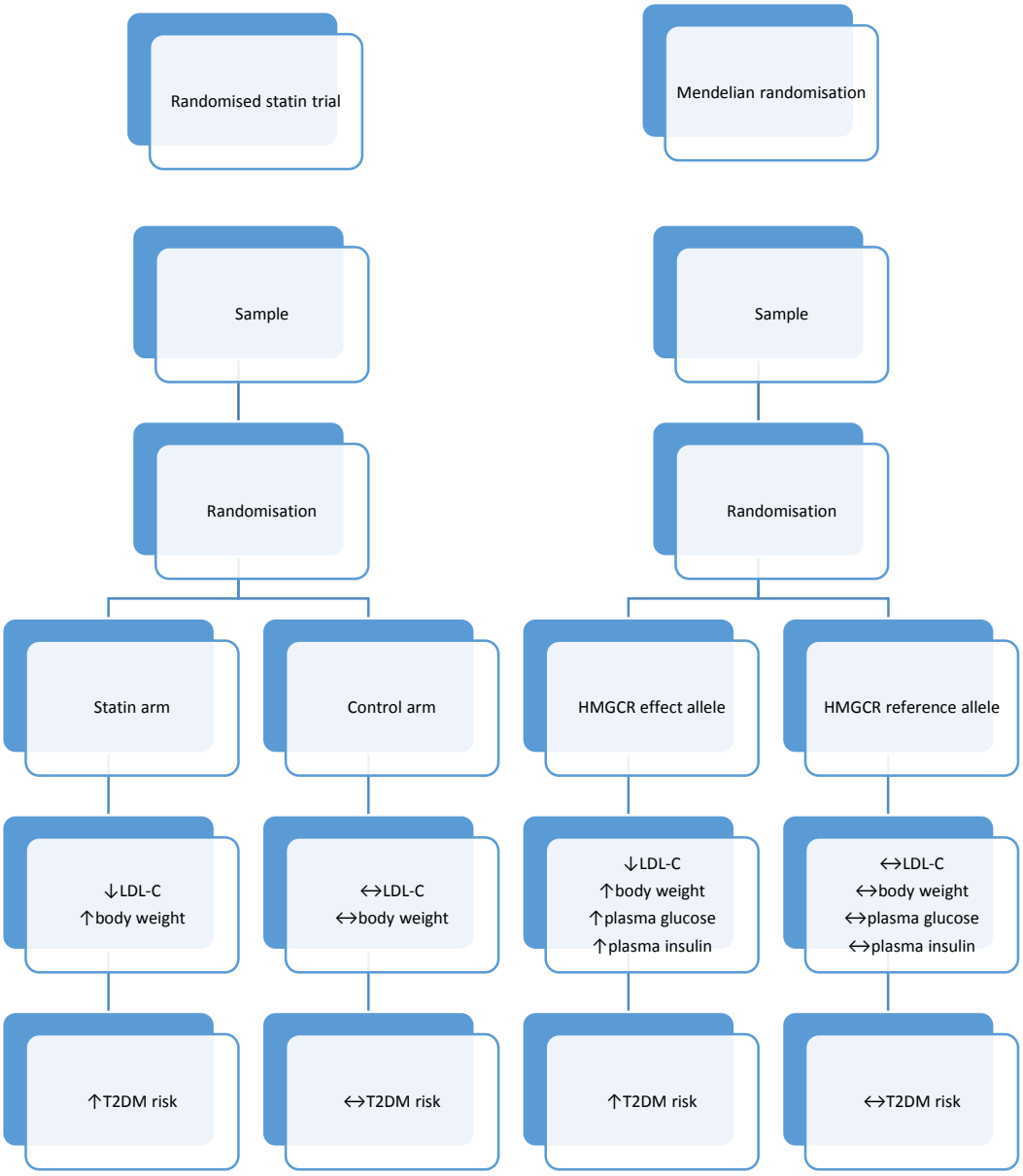

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MS Number	MOL270208
Corresponding Author name	David Preiss
Review title	**Genetic insights into statin-associated diabetes risk**
Section	Genetics & Molecular Biology
Author address on MS?	Y
Author phone number?	Y
Author email (if not on MS)	Y

Structured abstract	Y
Key words (max. 5)	Y
Introduction	Y
Headings in text	Y
Conclusion	Y
Key points box	Y

Word count: abstract	196
Word count: text	2350

Bullets and annotations/ within RP?	Y
Refs. in sequence?	Y
Acknowledgements; Financial support and sponsorship; Conflicts of interest.	Y/Y/Y

Vancouver references?/Page numbers

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