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SHORT COMMUNICATION

The loss-of-function *PCSK9* p.R46L genetic variant does not alter glucose homeostasis

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Abstract

Aims/hypothesis Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a critical regulator of cholesterol homeostasis. PCSK9 inhibitors are being actively developed to lower LDL-cholesterol levels. However, there are conflicting data regarding the consequences of *Pcsk9* deficiency on glucose homeostasis in mouse models. Here, we analysed in humans the association between the *PCSK9* p.R46L loss-of-function (LOF) variant and (1) glucose homeostasis variables; (2) type 2 diabetes status; and (3) the risk of 9 year incident type 2 diabetes in a prospective study.

Methods *PCSK9* p.R46L was genotyped in 4630 French participants from the Data from an Epidemiological Study on the Insulin Resistance Syndrome (DESIR) prospective study and in 1342 French participants with type 2 diabetes. The association between p.R46L and metabolic traits or type 2 diabetes risk was assessed through linear or logistic regression models adjusted for age, sex and BMI. The association between p.R46L and incident type 2 diabetes was assessed using a Cox regression model adjusted for sex, age and BMI at baseline.

Results Significant associations ($p < 10^{-6}$) were found between p.R46L and lower total cholesterol (−0.394 mmol/l), LDL-cholesterol (−0.393 mmol/l) and apolipoprotein B concentrations (−0.099 g/l). However, no significant association was observed between p.R46L and markers of glucose homeostasis (including fasting glucose, fasting insulin, HbA_{1c}, HOMA-B, HOMA-IR) or type 2 diabetes risk. Furthermore, no significant association between p.R46L variant and risk of incident type 2 diabetes was observed in DESIR.

Conclusions/interpretation The *PCSK9* p.R46L LOF variant was not associated with impaired glucose homeostasis in

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Statistical analyses The associations between p.R46L and quantitative traits was assessed in 4319 non-diabetic participants from DESIR through linear regression models adjusted for age, sex and BMI (except for the analysis of BMI, which was adjusted for age and sex only).

The case–control analysis included type 2 diabetes participants from DESIR (at baseline) and from Corbeil, and normal glucose controls from DESIR (during the follow-up period). The association between p.R46L and type 2 diabetes was assessed using a logistic regression model adjusted for age, sex and BMI.

The association between p.R46L and incident type 2 diabetes was assessed in DESIR using a Cox regression model adjusted for sex, age and BMI at baseline, and the timescale used was age.

All the analyses were performed under an additive model.

Given the size of our studies, we would have been able to detect a significant OR of 1.55 (type 2 diabetes case–control study) and a significant HR of 4.40 (incident type 2 diabetes study), with a power of 80% (ESM Fig. 2).

Except for statistical power analyses, we performed statistical analyses using SPSS (version 14.0), IBM, Armonk, NY, USA.

Results

In the 4,319 non-diabetic participants from the DESIR study, we assessed the association of *PCSK9* p.R46L with BMI, glucose homeostasis related traits (including FPG, FSI, HOMA-B, HOMA-IR and, HbA_{1c}), lipid homeostasis variables (including total cholesterol, HDL-cholesterol, LDL-cholesterol, triacylglycerol, apolipoprotein B [APOB] and apolipoprotein A1 [APOA1]) and liver enzyme levels (alanine aminotransferase and aspartate aminotransferase [Table 1]). When we adjusted our models for age, sex and BMI, we confirmed a marked association between the p.R46L variant and decreased total cholesterol levels (effect size [95% CI] −0.394 [−0.537, −0.251] mmol/l; $p=7.05\times10^{-8}$), decreased LDL-cholesterol levels (effect size −0.393 [−0.525, −0.261] mmol/l; $p=5.18\times10^{-9}$) and decreased APOB levels (effect size −0.099 [−0.136, −0.061] g/l; $p=2.42\times10^{-7}$ [Table 1]). These association results were similar when the models were adjusted for age, sex, BMI and lipid-lowering medication (data not shown). However, we did not find any significant association between p.R46L and variations in other traits (Table 1). According to the Meta-analyses of Glucose and Insulin-Related Traits Consortium (MAGIC), in >133,000 non-diabetic participants, p.R46L was nominally (but not significantly) associated with increase fasting glucose levels (effect size 0.028 [−0.006, 0.050] mmol/l; $p=0.013$), and it was not associated with fasting insulin levels (effect size 0.016 [−0.008, 0.040] pmol/l; $p=0.16$) [10].

Of note, in the 4,319 non-diabetic participants from DESIR, there was no significant difference regarding statin use between p.R46L carriers (n=4 of 171 [2.3%] participants informed for statin use) and non-carriers (n=108 out of 4,082 [2.6%] participants informed for statin use; $p=0.81$).

In the type 2 diabetes case–control study, we did not find any significant association between p.R46L and type 2 diabetes ($p=0.261$) or incident type 2 diabetes over the 9 years of follow-up ($p=0.065$ [Table 2]). In addition, there was no significant association between p.R46L and occurrence of impaired fasting glucose (FPG ≥6.1 mmol/l; n=508 incident cases) over the 9 years of follow-up (HR [95% CI] 0.91 [0.58, 1.42]; $p=0.66$; data not shown). According to the Diabetes Genetics Replication and Meta-analysis (DIAGRAM) consortium, p.R46L was not associated with type 2 diabetes risk in a total of 42,590 European participants (OR [95% CI] 1.09 [0.98, 1.22]; $p=0.12$) [11].

Discussion

The major finding of this study is that the *PCSK9* p.R46L LOF variant was not associated with impaired glucose homeostasis in humans. Notably, we showed that p.R46L carriers did not have an increased incidence of type 2 diabetes during a 9 year follow-up or type 2 diabetes risk in a case–control study.

Although positive correlations were observed between plasma PCSK9 and both FPG and HOMA-IR in the Dallas Heart Study [12], we did not find any associations between p.R46L and either FPG, HbA_{1c}, or markers of insulin resistance (HOMA-IR) or insulin secretion (HOMA-B). However, a non-clinically relevant tendency for a positive association between p.R46L and FPG was observed in MAGIC. As expected [1, 2], p.R46L remained associated with markers of LDL metabolism with reduced total cholesterol, LDL-cholesterol and APOB concentrations in people carrying the p.R46L allele. The use of statins is associated with an increased risk of type 2 diabetes and could be a potential confounding factor [13]. However, adding lipid-lowering medication status into the model did not modify the results.

The present results are in line with our previous results obtained in *PCSK9*-deficient mice, in which there was no alteration of glucose homeostasis, as assessed by both glucose and insulin tolerance tests [5]. While *PCSK9* deficiency is associated with an increased expression of LDLR in pancreatic islets, there is no increase in intracellular cholesterol content in *PCSK9*-deficient islets, even under lipotoxic conditions when incubating isolated islets with high concentrations of LDL [5]. This absence of intracellular cholesterol accumulation might be due to reduced circulating LDL levels in *PCSK9*-deficient mice and/or to an increase in cholesterol efflux from beta cell.

Table 1 Associations between the *PCSK9* p.R46L variant and glucose homeostasis related traits, lipid homeostasis variables and liver enzyme levels in 4,319 non-diabetic participants from DESIR

Clinical data	n	Mean/median data level by genotype[a]		Effect size (95% CI)	p value
		GG	GT		
Sex (M/F)	2,073 / 2,246	1,980 / 2,166	93/80	–	–
Age (years)	4,319	46.7±10.0	47.9±10.1	–	–
BMI (kg/m^2)	4,253	24.5±3.7	24.7±3.7	0.046 (−0.49, 0.58)	0.865
FPG (mmol/l)	4,313	5.24±0.55	5.29±0.54	0.013 (−0.026, 0.052)	0.736
FSI (pmol/l)	4,316	38.9 (28.5–54.7)	39.6 (28.0–59.6)	0.006 (−0.062, 0.074)	0.864
HOMA-B	4,310	67.4 (48.9–95.1)	68.2 (46.3–95.9)	−0.003 (−0.040, 0.033)	0.927
HOMA-IR	4,310	1.30 (0.92–1.90)	1.30 (0.92–2.04)	0.008 (−0.030, 0.046)	0.833
HbA$_{1c}$ (%)	4,314	5.25±0.41	5.28±0.39	0.008 (−0.050, 0.066)	0.788
HbA$_{1c}$ (mmol/mol)	4,314	33.9±4.5	34.2±4.3	0.086 (−0.543, 0.716)	0.788
TC (mmol/l)	4,319	5.74±1.00	5.39±0.95	−0.394 (−0.537, −0.251)	7.05×10^{-8}
HDL-C (mmol/l)	4,278	1.65±0.43	1.63±0.42	−0.004 (−0.064, 0.055)	0.890
LDL-C (mmol/l)	4,278	3.58±0.92	3.24±0.87	−0.393 (−0.525, −0.261)	5.18×10^{-9}
TG (mmol/l)	4,319	0.93 (0.67–1.35)	0.98 (0.67–1.49)	0.012 (−0.061, 0.085)	0.744
APOB (g/l)	4,318	1.09±0.27	1.00±0.26	−0.099 (−0.136, −0.061)	2.42×10^{-7}
APOA1 (g/l)	4,318	1.65±0.26	1.64±0.24	−0.001 (−0.039, 0.036)	0.942
ALAT (U/l)	4,316	21.3 (15.8–29.5)	21.8 (15.8–31.5)	0.024 (−0.041, 0.089)	0.474
AST (U/l)	4,316	18.6 (15.0–23.2)	18.6 (15.8–22.9)	−0.011 (−0.063, 0.041)	0.689

[a] Data are means ± SD or, for logarithmically transformed variables (including FSI, HOMA-B, HOMA-IR, TG, ALAT and AST), data are median (25th–75th percentile)

ALAT, alanine aminotransferase; AST, aspartate aminotransferase; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; TC, total cholesterol; TG, triacylglycerol

Table 2 Association between the *PCSK9* p.R46L variant and type 2 diabetes risk and incidence

	n	T-allele frequency	GG	GT	TT	OR or HR (95% CI)	p value
Type 2 diabetes case–control analysis							
Normal glucose controls from DESIR	2,207	2.15%	2,112	95	0	0.81 (0.56–1.17)[a]	0.261
Type 2 diabetes cases from Corbeil study and DESIR	1,469	1.97%	1,412	56	1		
Type 2 diabetes incidence analysis							
Non-diabetic participants from DESIR	4,280	2.09%	4,108	172	0	0.34 (0.11–1.07)[b]	0.065
Incident type 2 diabetes cases from DESIR	184	0.83%	181	3	0		

Data are n unless otherwise indicated

[a] OR from a logistic regression model adjusted for age, sex and BMI

[b] HR from a Cox regression model adjusted for age, sex and BMI at baseline

Our study has several limitations that should be highlighted. First, we only focussed on the effect of the most frequent *PCSK9* LOF variant, i.e. p.R46L, and we cannot exclude the possibility that other rare *PCSK9* LOF mutations with stronger functional effects may alter glucose homeostasis. Second, the intensity of PCSK9 inhibition observed in heterozygous carriers of the p.R46L variant is inferior to those obtained with PCSK9 monoclonal antibodies. Indeed, the decrease of LDL-cholesterol is 10% in p.R46L carriers compared with ~60% with alirocumab or evolocumab in Phase 2 trials [4]. However, such a small decrease in LDL-cholesterol was consistently associated with a highly significant reduction of cardiovascular events in individuals carrying the p.R46L allele [1, 2], certainly because it was maintained throughout their lives.

In conclusion, a genetic *PCSK9* deficiency is not associated with an increased risk of type 2 diabetes. These data are reassuring regarding the safety of PCSK9 inhibitors. Precise glucose phenotyping of individuals treated with PCSK9 monoclonal antibodies in ongoing Phase 3 trials will give some additional insights into the relationship between PCSK9 and glucose homeostasis.

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