T-cadherin gene variants are associated with type 2 diabetes and the Fatty Liver Index in the French population

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Abbreviations

BMI, body mass index; CI, confidence Interval; FLI, fatty liver index; GGT, gamma-glutamyl-transferase; GWAS, genome-wide association study; HMW, high molecular weight; LMW, low molecular weight; MAF, minor allele frequency; MMW, medium molecular weight; NAFLD, non-alcoholic fatty liver disease; OR, odds-ratio; TCAD, T-cadherin

Abstract

Aim

Adiponectin is an adipocyte-secreted protein associated with insulin sensitivity. T-cadherin is a receptor for high and medium molecular weight adiponectin. In GWAS, T-cadherin gene (*CDH13*) polymorphisms are associated with circulating adiponectin levels. We investigate the associations between genetic variants of *CDH13* and type 2 diabetes, and related parameters, in a Caucasian population.

Methods

Two polymorphisms of *CDH13* (rs11646213 and rs3865188) were genotyped in French cohorts, firstly in a general population D.E.S.I.R. (N=5212), and secondly in people with type 2 diabetes, DIABHYCAR (N=3123). Adiponectin levels at baseline were measured in D.E.SI.R. participants who were normoglycaemic at baseline but hyperglycaemic after 3 years (n=230) and controls who remained normoglycaemic (n=226).

Results

In a cross sectional analysis, *CDH13* genotype distributions differed between people with and without type 2 diabetes with odds-ratio (95% CI) for type 2 diabetes of 1.11 (1.04-1.18) (p=0.001), and 0.92 (0.87-0.98) (p=0.01) for rs11646213 and rs3865188, respectively. The rs11646213 variant, associated with a higher odds-ratio for type 2 diabetes, was also associated with higher BMI (p=0.03), HbA1c (p=0.006) and lower plasma adiponectin levels (p=0.03) in the D.E.S.I.R. general population. Conversely, the variant rs3865188, associated with a lower odds-ratio for type 2 diabetes, was also associated with lower BMI (p=0.03), HbA1c (p=0.02) and fatty liver index ($p \leq 0.01$) and higher plasma adiponectin (p=0.002). The associations with HbA1c, fatty liver index and adiponectin levels persisted after adjustments on BMI.

Conclusion

CDH13 polymorphisms were associated with prevalent type 2 diabetes in this French population. The association may be mediated through effects on BMI and/or plasma adiponectin.

Keywords

T-cadherin; genetic polymorphisms; adiponectin; type 2 diabetes; body mass index; fatty liver index

Introduction

Adiponectin is an adipokine mainly secreted by adipocytes. Adiponectin levels have been associated with type 2 diabetes and various related traits, including non-alcoholic fatty liver disease (NAFLD) [1–4].

It circulates in the blood in a number of complexes which include trimers described as low-molecular weight (LMW) oligomers, hexamers as medium-molecular weight (MMW), and high-molecular weight (HMW) multimers (12-, 18-mers and possibly larger). The HMW isomer is the most abundant and has been described as responsible for the beneficial effects of adiponectin. Adiponectin acts through two main receptors AdipoR1, AdipoR2 and an additional receptor, T-cadherin, which binds only to MMW and HMW adiponectin [5].

T-cadherin, unlike other cadherins, is not responsible for cell adhesion but participates in intracellular signal transmission [6]. It has been demonstrated that T-cadherin is essential for the cardioprotective effect of adiponectin [7], and has been associated with insulin secretion [8].

In Genome Wide Association Studies (GWAS), T-cadherin gene (*CDH13*) polymorphisms were associated with circulating adiponectin levels [9,10]. *CDH13* polymorphisms were also associated with the risk of the metabolic syndrome and blood pressure in Europeans [11,12] and type 2 diabetes in Asian men [13]. In addition, a meta-analysis showed that a genetic score, including *CDH13*, associated with adiponectin levels, might influence the risk of type 2 diabetes in a multiethnic population [9].

In our previous studies on the D.E.S.I.R. cohort, we have found that low adiponectin levels were associated with a higher risk of hyperglycemia and type 2 diabetes. A low fatty liver index (FLI), surrogate marker of liver steatosis, was associated with a lower risk of the incidence of type 2 diabetes at the 9-year follow-up [14]. The aim of the present study was to

test the associations of *CDH13* polymorphisms with adiponectin levels and type 2 diabetes risk, as well as related metabolic traits including the FLI, in the French D.E.S.I.R. study population. We also performed a case-control comparison between people without type 2 diabetes in D.E.S.I.R. and the people with type 2 diabetes from the D.E.S.I.R. and DIABHYCAR cohort.

Material and Methods

Participants

Two populations were included: volunteers from the general French population in D.E.S.I.R. and patients with type 2 diabetes in DIABHYCAR.

The D.E.S.I.R. study is a prospective study that included 2,576 men and 2,636 women, aged 30–65 years, recruited from volunteers who were offered periodic health examinations free of charge by the French Social Security system in 10 health examination centers from the western part of France. They were clinically and biologically evaluated at visits every 3 years, and the final examination was 9 years after inclusion. The study was approved by the ethics committee of the Kremlin Bicêtre Hospital, and all participants signed an informed consent. The clinical and biological measurements have been extensively previously described [15].

The DIABHYCAR was a 6-year clinical trial in men and women with type 2 diabetes and included 3,137 unrelated French people. The trial tested whether a low dose of Ramipril, an ACE inhibitor, would reduce cardiovascular and/or renal events. Negative results from this study were published previously [16,17]. Participants gave written informed consent and study protocols were approved by the Ethics Committee of Angers University Hospital.

Characteristics of the D.E.S.I.R. and DIABHYCAR cohorts are presented in Table 1.

Adiponectin Assay Measurement

In D.E.S.I.R., adiponectin levels were measured by radioimmunoassay (RIA) at baseline, in 456 people with baseline normoglycaemia (fasting plasma glucose < 6.1 mmol/l); 226 people who remained normoglycaemic, were matched for sex, age and BMI with the 230 who became hyperglycaemic (fasting plasma glucose ≥ 6.1 mmol/l) at 3 years [1,18].

Fatty Liver Index

The FLI, a predictor of fatty liver in the general population, is based on an algorithm that includes Body Mass Index (BMI), triglycerides, Gamma-Glutamyl-Transferase (GGT) and waist circumference [19]. In the present study, we dichotomized the FLI into 2 categories, over 70 and under 70, because a FLI value over 70 predicted the onset of type 2 diabetes in our previous study [14].

Genotyping

Two single nucleotide polymorphisms (SNP) of *CDH13* were selected on the basis of previous studies. These SNPs (rs11646213 [A>T] and rs3865188 [A>T]) have been associated with plasma adiponectin levels in GWAS [10,20,21]. They are located in the gene promoter.

All polymorphisms were genotyped in the whole population using Kaspar method by LGC Genomics, Hoddesdon, UK (http://www.lgcgenomics.com). The genotyping success rate was 0.97 for both in D.E.S.I.R. and 0.96 (rs11646213) and 0.97 (rs3865188) in DIABHYCAR.

Statistical Analysis

The associations between the genotypes and continuous variables were estimated using ANCOVA for repeated measures, with adjustments for confounding factors (age, sex, BMI). Parameters that were not normally distributed (for example the adiponectin levels) were Log transformed before analysis. Associations between baseline adiponectin levels and continuous

variables were assessed by linear regression, adjusting for confounding variables (sex, age, glycemic status, BMI). Association between baseline adiponectin levels and 9-year prevalence of hyperglycemia was tested by ANCOVA adjusting for sex, age and BMI. Associations between polymorphisms and metabolic diseases used logistic regression, adjusted for confounding factors, and quantified by Odds Ratios (OR) with 95% confidence intervals (CI). Correction for multiple comparisons due to multiple SNP testing took into account the effective number of independent tests (Meff) based on the degree of linkage disequilibrium between SNPs [22]. A value of $p \leq 0.033$ was considered significant. Departure from the Hardy Weinberg equilibrium was assessed using a χ^2 test. P values were 0.04 and 0.17 (rs11646213) and 0.07 and 0.04 (rs3865188) in D.E.S.I.R. and DIABHYCAR, respectively. The tests concerning polymorphisms correspond to the best fitting models of inheritance according to descriptive statistics (additive, dominant or recessive).

Statistical analyses used SYSTAT 13® for Windows.

In the D.E.S.I.R. study, we excluded people born outside mainland France in the genetic analyses. For analyses with the FLI, we only studied the 3650 people with alcohol consumption levels lower than 30 g/day (men) and 20 g/day (women).

Results

D.E.S.I.R. Cohort

Baseline adiponectin levels were negatively and significantly associated with BMI (p=0.02) and FLI (p<0.0001) and this latter association remained after further adjustment for BMI (p=0.0006). There was no association with HbA1c (p=0.23). Plasma adiponectin was also associated with the prevalence of hyperglycaemia at 9 years (ANCOVA adjusted for sex, age and BMI: p=0.01).

The rs11646213 A allele was significantly associated with higher BMI and HbA1c (*Table 2*) as well as with lower plasma adiponectin levels (*Table 3*) (adjusted on age, sex and BMI, when appropriate). In people who reported at baseline to drink no alcohol or who had a light alcohol consumption, at the end of the study, the A allele was more frequent in those with FLI≥70 (OR [95% CI]: 1.22 [1.00 - 1.49]); but this difference did not reach the statistical threshold of significance (p=0.05) (*Table 4*).

The rs3865188 A allele was significantly associated with lower BMI and HbA1c (*Table 2*), and higher plasma adiponectin levels (*Table 3*). The association with HbA1c was no longer significant after adjusting for BMI. It was also associated with a lower risk of being in the FLI≥70 group at baseline (OR [95% CI]: 0.72 [0.57 – 0.92]; p=0.01) and at the end of the study (OR [95% CI]: 0.75 [0.61 – 0.92]; p=0.005) (*Table 4*).

Association with Type 2 Diabetes

The genotypes and allele frequencies were similar between the people with type 2 diabetes in D.E.S.I.R. and DIABHYCAR. In order to increase the power of the statistical analysis, we performed a case-control comparison between people without type 2 diabetes in D.E.S.I.R. and the people with type 2 diabetes from DESIR and DIABHYCAR (Table 5). The two polymorphisms were significantly associated with type 2 diabetes, with a higher risk for the minor allele of rs11646213 (OR 1.11, 95% CI: 1.04-1.19, p = 0.001) and a lower risk for the minor allele of rs3865188 (OR 0.92, 95% CI: 0.87-0.99, p = 0.01).

Discussion

Our main results in the D.E.S.I.R. and DIABHYCAR cohorts show associations of two polymorphisms of *CDH13* with type 2 diabetes, as well as with BMI, FLI and circulating adiponectin levels.

The two variants were associated with the prevalence of type 2 diabetes in a case-control study including people without type 2 diabetes from D.E.S.I.R. and people with type 2 diabetes from D.E.S.I.R. and DIABHYCAR cohorts. Consistent with this association, in D.E.S.I.R., the type 2 diabetes at-risk alleles were also associated with higher HbA1c levels. To our knowledge, this is the first reporting of an association between *CDH13* variants and the risk of type 2 diabetes in a European Caucasian population. However, in Swedes, rs11646213 was associated with the metabolic syndrome [11] and in East Asian populations, rs3865188 was associated with metabolic parameters, in the same directions as in our study [13,23,24]. The mechanisms behind these associations are unclear.

For both polymorphisms, the variants at risk for type 2 diabetes in our study were also associated with lower adiponectin levels. This is consistent with the insulin-sensitizing role of adiponectin[25–27]. Circulating adiponectin levels are negatively associated with the development of type 2 diabetes [28]. Nevertheless, some data concerning the role of t-cadherin in adiponectin action are not consistent with this straightforward explanation. T-cadherin acts as a co-receptor for HMW and MMW adiponectin [7]. In the heart, Denzel *et al.* showed that t-cadherin was essential for adiponectin-mediated activation of the AMP activated protein kinase signaling pathway. In the same study, plasma adiponectin levels increased in TCAD-KO mice. Despite high circulating adiponectin levels, these animals were not protected from ischemia-reperfusion cardiac injuries. This phenomenon can be interpreted as an adiponectin resistance caused by the absence of t-cadherin necessary for tissue uptake of adiponectin. Accordingly, in our study, the *CDH13* alleles associated with high adiponectin levels should also be associated with adiponectin resistance. Since our findings show that higher levels are concomitant with a lower risk of type 2 diabetes, it indicates that this t-cadherin induced adiponectin resistance does not affect the susceptibility to type 2 diabetes as it does with stress induced cardiac injuries. Actually, in agreement with the adiponectin

resistance hypothesis, Gao *et al.* found that CDH13 alleles associated with high circulating adiponectin levels associate with a deleterious metabolic profile [24]. These results are discordant with our data. The difference with our study might be explained by the European origin of the population. Differences in the genetic architecture within the *CDH13* locus between Europeans and Asians have been described [29]. Another difference comes from the recruitment procedure. In fact, D.E.S.I.R. is a population-based study while the above-mentioned studies included many patients with metabolic diseases where adiponectin resistance might be occurring, therefore inhibiting the protective effect of adiponectin. On a molecular basis, Putku *et al.* stated that the *CDH13* promotor region holds several methylated quantitative trait loci (meQTLs) that they found to be associated with cardiovascular traits [30]. The polymorphisms that we studied are localized in the promoter region harboring those meQTLs and are in linkage disequilibrium with four SNPs exhibiting effect on methylation level. The association of the SNPs we studied with certain metabolic traits might therefore depend on the promoter methylation status that varies across populations, with different lifestyles and genetic backgrounds. Further studies will be necessary to determine whether these SNPs are directly functional or whether they are only linked to causal variants which might be meQTLs.

In the present study, one *CDH13* alleles associated with a higher relative risk of type 2 diabetes was also associated with high FLI levels, previously found predictive of type 2 diabetes. Therefore, *CDH13* alleles might be associated with type 2 diabetes through the liver profile. In the absence of hepatocarcinoma, there has been no evidence of t-cadherin expression in hepatocytes [31]. Therefore, the association with type 2 diabetes may be secondary to the association with plasma adiponectin levels. Adiponectin has antisteatotic and antiapoptotic effects on hepatocytes [32]. Adiponectin levels are associated with NAFLD [3,33], and with FLI in the present study. It has been proposed that fatty liver may induce

insulin resistance [34]. Therefore, *CDH13* might influence type 2 diabetes risk by an effect on adiponectin levels and also by an effect on fatty liver. Nevertheless, since the effects on fatty liver likely depend on the effect on adiponectin, these mechanisms are likely closely linked. The connection between these effects may be the ceramide pathway. It has been suggested that ceramides which are associated with insulin resistance play a major role in NAFLD progression [35]. Adiponectin diminishes the accumulation of ceramides by activating ceramidase activity of receptors AdipoR1/R2 [36]. It has been suggested that this latter effect was central to most of the adiponectin actions [36].

The *CDH13* variants were also associated with BMI. In East Asian populations, a similar result has sometimes been found [21,24]. In a recent large meta-analysis of GWAS data on BMI [37], a *CDH13* variant (rs8062451) was associated with BMI ($p=7.33x10^{-6}$). The mechanism of such an association is not known. In our study, this association is in line with the inverse relationship between BMI and adiponectin levels. Nevertheless, when adjusting for BMI, the association with adiponectin persists. The associations between *CDH13* alleles and HbA1c and FLI also persisted after adjustments on BMI, which indicates the independence, at least partial, of these associations. T-cadherin might therefore have independent pleiotropic effects. Actually T-cadherin and plasma adiponectin levels might independently affect the development of type 2 diabetes.

Our study has several limitations. We could not adjust our genetic analyses for population stratification. Nevertheless, only people born in mainland France were analyzed, which should limit the ethnic heterogeneity. Due to the low number of adiponectin measures within the population, we could not adjust our results for plasma adiponectin levels. This adjustment would have allowed us to describe the role of adiponectin in the associations between *CDH13* and some phenotypes such as HbA1c. We could not show genetic associations with FLI as a continuous variable. Therefore, since our result is described for the first time, this association

needs to be replicated. Also, concerning the association with type 2 diabetes, the D.E.S.I.R. study alone lacked statistical power, as it included only 294 people with type 2 diabetes. Nevertheless, we showed associations in this cohort of the two *CDH13* polymorphisms with HbA1c and the FLI threshold predictive of type 2 diabetes incidence, consistent with the case control comparison between D.E.S.I.R. and DIABHYCAR.

In conclusion, the two common polymorphisms of *CDH13* are associated with type 2 diabetes, FLI, BMI and circulating adiponectin. Further studies are needed to clarify the mechanisms of these associations in order to determine whether these results indicate new pathways in the pathophysiology of type 2 diabetes and a possible new therapeutic target.

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Table 1. Characteristics at inclusion in D.E.S.I.R and DIABHYCAR

Clinical data	D.E.S.I.R.	DIABHYCAR
N (% men)	5212 (50%)	3123 (73%)
Age (years)	47 ± 10	65± 8
BMI (kg/m^2)	24.7 ± 3.8	29.4 ± 4.6
Waist circumference (cm)	83 ± 12	-
Glucose (mmol/l)	5.4 ± 0.8	9.6 ± 3.1
HbA1c (%)	5.3 ± 0.5	7.9 ± 1.8
Insulin (pmol/l)	39.8 (27.8-57.3)	-
Fatty Liver Index ≥70 (%)	11.2	
Total Cholesterol (mmol/l)	5.73 ± 1.02	5.79 ± 1.07
HDL-C (mmol/l)	1.62 ± 0.42	1.32 ± 0.36
LDL-C (mmol/l)	3.36 ± 1.02	3.53 ± 0.88
Triglycerides (mmol/l)	0.97 (0.68-1.43)	1.83 (1.30-2.67)
Diastolic blood pressure (mm Hg)	80 ± 10	82 ± 8
Systolic blood pressure (mm Hg)	131 ± 16	144 ± 13

BMI, body mass index;
Data are N (percentage), means ± SDs, medians (quartile 1, quartile 3)

Table 2. Association between the CDH13 rs11646213, rs3865188 variants and metabolic traits in the D.E.S.I.R. study.

Polymorphism		N	BMI (kg/m^2)		HbA1c (%)	
			T0	T9	T0	T9
rs11646213	TT	1615	24.6 ± 0.1	25.5 ± 0.12	5.43 ± 0.01	5.58 ± 0.02
	TA	2170	24.7 ± 0.08	25.8 ± 0.1	5.47 ± 0.01	5.62 ± 0.01
	AA	827	24.9 ± 0.13	26.0 ± 0.16	5.48 ± 0.02	5.64 ± 0.02
p-value *			0.03		0.006/0.02	
rs3865188	TT	1383	24.8 ± 0.1	25.9 ± 0.12	5.47 ± 0.01	5.62 ± 0.02
	TA	2220	24.7 ± 0.08	25.8 ± 0.1	5.47 ± 0.01	5.62 ± 0.01
	AA	993	24.5 ± 0.12	25.4 ± 0.15	5.43 ± 0.02	5.58 ± 0.02
p-value *			0.03		0.02/0.09	

Data shown are mean ± SD at inclusion (T0) and at 9-year follow-up (T9)

*p-value of genotype effect by ANCOVA for repeated measures adjusted for sex, age (/ + BMI), when appropriate. Additive model was used except for rs3865188 in HbA1c (recessive model analysis). No significant interactions genotype x time were found.

Table 3. Plasma adiponectin levels according to CDH13 genotypes in a subsample of the D.E.S.I.R. cohort.

Polymorphism		N	Adiponectin (µg/mL)
rs11646213	TT	160	23.6 (15.2-35.9)
	TA	191	24.0 (14.9-38.9)
	AA	67	21.1 (12.8-34.8)
p-value *			0.03/0.008
rs3865188	TT	132	22.2 (13.5-36.6)
	TA	185	23.8 (15.0-37.7)
	AA	99	24.5 (16.3-37.0)
p-value*			0.002/0.001

Data show are medians (quartile 1, quartile 3) at T0.
p-value of genotype effect (additive model groups), adjusted for age, sex, BMI and glycemic status / idem + *ADIPOQ* polymorphisms

Table 4. Association between the CDH13 rs11646213 and rs3865188 variants and FLI. The D.E.S.I.R. Study

			At baseline, T0				**At 9 year follow-up, T9**			
			FLI (%)		p-value	OR (95% IC)*	FLI (%)		p-value*	OR (95% IC)*
		N	< 70	≥ 70			< 70	≥ 70		
rs11646213	TT	1225	35.4	32.2			35.8	29.8		
	TA	1625	46.6	47.0	0.35	1.12 (0.88 – 1.43)	46.4	47.9	0.05	1.22 (1.00 – 1.49)
	AA	635	18.0	20.8			17.8	22.3		
	MAF		41.3	44.3			41.0	46.3		
rs3865188	TT	1074	30.3	37.6			30.3	37.1		
	TA	1649	47.5	45.9	0.010	0.72 (0.57 – 0.92)	47.5	48.4	0.005	0.75 (0.61 – 0.92)
	AA	759	22.2	16.5			22.2	14.5		
	MAF		46.0	39.5			46.0	38.7		

Data shown are percentages within FLI classes < 70 and ≥ 70;

* p-value and odds-ratios for the minor allele by logistic regression with recessive model for rs11646213 (AA vs. Tx) and dominant model for rs3865188 (TT vs. Ax), adjusted for sex, age, BMI and alcohol consumption.

MAF: minor allele frequency

Table 5. Genotype and allele frequencies of CDH13 *polymorphisms according to type 2 diabetic status in the DIABHYCAR and D.E.S.I.R. studies*

Polymorphism	Cases from DIABHYCAR+DESIR	Controls from D.E.S.I.R.	p-value*	OR (95% IC)*
rs11646213				
TT	1055 (32.0)	1521 (35.2)	0.001	1.11 (1.04-1.18)
TA	1581 (48.0)	2026 (46.9)		
AA	656 (19.9)	769 (17.8)		
rs3865188				
TT	1075 (32.2)	1294 (30.1)	0.01	0.92 (0.87-0.98)
TA	1593 (47.8)	2066 (48.0)		
AA	666 (20.0)	941 (21.9)		

Data shown are N (%)

**p*-value and odds-ratios for the minor allele by logistic regression (additive models)