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SHORT CONTRIBUTION

ADIPOQ gene polymorphism rs1501299 interacts with fibre intake to affect adiponectin concentration in children: the GENe–Diet Attica Investigation on childhood obesity

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Abstract
Background Adiponectin, an adipose-derived hormone with central and peripheral actions, is involved in the regulation of energy homeostasis. Interactions between genetic and environmental factors have been associated with decrease in circulating adiponectin leading to obesity.
Aim We investigated whether variants of the *ADIPOQ* gene encoding adiponectin interact with diet to predict serum adiponectin concentration.
Methods A cross-sectional study of healthy school-aged children of Greek origin ($n = 991$), aged 11.2 ± 0.6 years was conducted in 2005–2006. DNA was genotyped for two SNPs [rs1501299 ($n = 741$) and rs17300539 ($n = 713$)] located in the *ADIPOQ* gene. Detailed dietary, behavioural, lifestyle, anthropometric and biochemical data were recorded for all participants.
Results Both SNPs were in HWE. The rs1501299 (GG vs GT + TT) × fibre interaction was significantly associated with adiponectin concentration ($P = 0.028$). When fibre intake was low, GG homozygotes exhibited significantly higher adiponectin concentrations compared to T allele carriers (mean ± SD = 5.1 ± 2.7 vs 4.2 ± 2.3; $P = 0.020$).
Conclusions In the present study, the rs1501299 × fibre interaction was significantly associated with adiponectin levels; in specific, GG homozygotes exhibited higher adiponectin levels compared to T carriers under conditions of lower fibre intake.

Keywords *ADIPOQ* gene SNP · Adiponectin · Children · Fibre · Gene–diet interactions

Abbreviations

BMI	Body mass index
SNP	Single nucleotide polymorphism
AMPK	AMP-activated protein kinase
PUFA	Polyunsaturated fatty acid
CVD	Cardiovascular disease
EI	Energy intake
BMR	Basal metabolic rate
HWE	Hardy–Weinberg equilibrium
SD	Standard deviation
MET	Metabolic cost of activity

Introduction

Adiponectin is an adipose-derived hormone, highly abundant in human plasma. Decreased adiponectin levels caused by interactions between genetic and environmental factors leading to obesity has been shown to contribute to the development of insulin resistance type 2 diabetes and metabolic syndrome [7].

The common G allele of the rs1501299 polymorphism located in intron 2 of adiponectin gene (*ADIPOQ*) has been associated with lower adiponectin levels in healthy Caucasians and obese children [1]. However, the T allele has

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been related to lower adiponectin in healthy Italians [2]. The G allele of polymorphism rs17300539, located in the promoter region of *ADIPOQ*, has been linked with lower adiponectin in obese and non-obese children [1].

Dietary factors have also been studied for their effects on adiponectin. In a study of healthy adolescents, macronutrient intake had no effect on adiponectin levels [15]. Diets, however, high in whole-grain cereals and dietary fibre have been positively associated with adiponectin levels in healthy women [16]. Moreover, diets low in glycemic load and high in cereal fibre intake were also reported to be positively associated with circulating adiponectin in diabetics [12].

Although the effects of either *ADIPOQ* gene variation or diet on adiponectin concentrations have been previously studied extensively, the way that *ADIPOQ* variants may interact with diet in affecting blood adiponectin levels has been much less studied, especially in children. Hence, the aim of the present study was to explore the effect of ADIPOQ gene polymorphisms (rs1501299 and rs17300539) and dietary intakes, as well as their interaction on serum adiponectin, in a population of healthy Greek children living in the area of Attica, who participated in the GENe–Diet Attica Investigation on childhood obesity (GENDAI).

Subjects and methods

The study population for the present analysis consisted of all study participants from the GENDAI who were of Greek origin [$n = 991$; mean age $\pm$ SD (years) 11.2 ± 0.6]. Anthropometric, biological and lifestyle characteristics were collected by trained personnel (paediatricians and dieticians), on the basis of a standard protocol. Details on the aims, methods and design of the GENDAI Study have been published elsewhere [9].

Dietary information was collected by two non-consecutive 24-h recalls; the second dietary recall was always conducted on a different day of the week from the first interview, 3–10 days after the first recall. The 24-h recall data were analysed using Nutritionist Pro software, version 2.2 (Axxya Systems-Nutritionist Pro, Stafford, TX, USA). The Nutritionist Pro food database was expanded by adding analyses of traditional Greek foods and recipes, and nutrient information for local processed food items (mainly snack foods, sweets and fast foods) as shared by the industry [9]. For the assessment of low energy reporting, the ratio of the EI/BMR was determined for each subject. Participants with EI/BMR ≤ 0.99 were classified as "low energy reporters" based on the cutoff limits developed by Goldberg et al. [3]. "Normal energy reporters" or "non-low energy reporters" were participants with EI/BMR ≥ 1.00.

Children were also categorized into pre-pubertal (Tanner I) and pubertal (Tanner II–IV) according to their Tanner stage. Post-pubertal (Tanner V) children ($n = 15$) and children whose pubertal status was not available ($n = 11$) were excluded from analyses.

Serum total adiponectin (μg/mL) was determined in one run via enzyme-linked immunosorbent assay using DuoSet human adiponectin ELISA (R&D Systems, Inc., Minneapolis, USA) on an automatic analyser (Sunrise ELISA Reader, TECAN). The sensitivity of analytics was 0.625 μg/mL. Two SNPs in the *ADIPOQ* gene rs1501299 and rs17300539 were genotyped using TaqMan Technology [13] (Applied Biosciences, ABI, Warrington, UK). Primers and MGB probes are available upon request.

Continuous variables are presented as mean values $\pm$ standard deviation. Associations amongst categorical variables were tested using the Chi-square test, whereas comparisons of mean values of normally distributed continuous variables between study groups were performed using analysis of variance (ANOVA). For non-normally distributed variables, we used the logarithmically transformed values. Correlations amongst study parameters were evaluated by calculation of the Pearson correlation coefficient for the normally distributed variables and by the Spearman correlation coefficient (r) for skewed variables. Multiple linear regression was performed to test the hypothesis of association between polymorphisms and adiponectin in response to macronutrient and dietary fibre intake after adjustment for potential confounders. General linear models were applied to test the association between adiponectin and SNPs and categories of fibre intake as fixed effect covariates after controlling for confounders. The level of significance was defined at $P \leq 0.05$. All statistical analyses were performed using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA). The power of the study was estimated with the use of Quanto 1.2 [4].

Results

No significant differences were observed in age, BMI, total adiponectin levels, protein, carbohydrate and total fat intake between boys and girls ($P > 0.05$) apart from MET score (boys 757.1 ± 423.1, girls 639.8 ± 468.6; $P < 0.001$), total energy intake (boys $2{,}032.3 \pm 624.3$, girls $1{,}769.8 \pm 562.9$ kcal/day; $P < 0.001$) and dietary fibre intake (boys 16.6 ± 9.5, girls 14.8 ± 8.3 g/day; $P = 0.003$). Adiponectin levels differed significantly based on pubertal status (pre-pubertal children 5.4 ± 2.9, pubertal children 4.6 ± 2.5 μg/mL; $P < 0.017$) and were significantly positively correlated with age (Spearman's $r = 0.188$; $P < 0.001$) and negatively with BMI (Spearman's $r = -0.125$; $P = 0.001$). Genotype distributions

did not deviate from Hardy–Weinberg equilibrium for both studied SNPs.

No significant associations were found between SNP genotypes and adiponectin or between macronutrient or dietary fibre intake and adiponectin, even after controlling for potential confounders. With regard to the gene–nutrient interactions, rs1501299 had no significant effect on adiponectin concentration in response to macronutrient intake (data not shown). However, the rs1501299 (GG vs GT + TT) × fibre interaction was significantly associated with adiponectin concentration ($P = 0.028$) (Table 1). When subjects were divided into tertiles according to their fibre intake, polymorphism had a significant effect ($P = 0.017$) on adiponectin only in the first tertile of fibre intake, which remained significant even after adjustment for confounders ($P = 0.020$). Specifically, GG homozygotes had significantly higher adiponectin levels compared to T carriers when fibre intake was low (5.1 ± 2.7 and 4.2 ± 2.3 μg/mL, respectively) (Fig. 1). With regard to rs17300539, no significant interactions were found with the dietary variables (data not shown). The observed power to test the effect of the interaction on adiponectin in the study group was 78.8%.

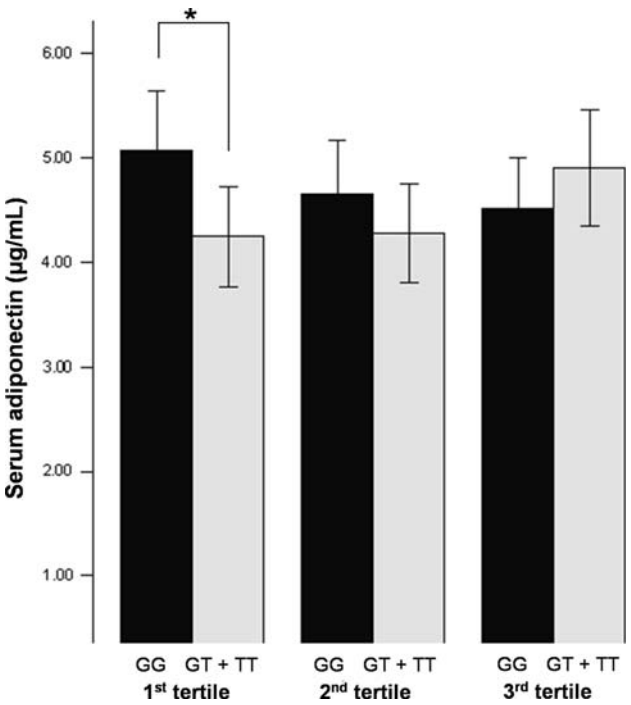

Fig. 1 Serum adiponectin concentration by rs1501299 genotype and fibre intake tertile. * Statistically significant difference between GG and GT + TT ($P = 0.017$) even after adjustment for confounders (gender, pubertal status, BMI, MET score, energy intake, low energy reporting) ($P = 0.020$)

Discussion

In the present cohort study, the influence of the adiponectin gene *ADIPOQ* polymorphisms (rs1501299 and rs17300539) on serum adiponectin concentration in response to dietary factors was investigated in healthy school-aged children. Overall, the rs1501299 × fibre interaction was significantly associated with adiponectin levels; in specific, GG homozygotes exhibited higher adiponectin levels compared to T carriers under conditions of lower fibre intake. Perez-Martinez et al. [10] also studied whether adiponectin gene variants interact with diet to affect insulin sensitivity in Caucasian men. Although they found no significant association for rs1501299 with insulin resistance in response to dietary fat consumption, they reported that homozygous subjects for C allele of rs16861194 were significantly less insulin resistant following consumption of a diet rich in MUFA and CHO compared with a diet rich in SFA.

In accordance with our findings, Yannakoulia et al. [15] reported no significant associations between macronutrient intake and circulating adiponectin in healthy adolescents.

Table 1 Effect of rs1501299 genotype × fibre intake interaction on adiponectin concentration (μg/mL)

	Core model		Core model + rs1501299 × fibre interaction	
	Beta ± SD	*P*	Beta ± SD	*P*
Gender	0.004 ± 0.221	0.906	0.023 ± 0.221	0.828
Pubertal status (pre-pubertal vs pubertal)	−0.550 ± 0.392	0.126	−0.567 ± 0.391	0.117
BMI (kg/m^2)	−0.093 ± 0.032	0.005	−0.096 ± 0.032	0.004
MET score	0.000 ± 0.000	0.053	0.000 ± 0.000	0.067
Total energy intake (kcal/day)	0.000 ± 0.000	0.188	0.000 ± 0.000	0.154
Underreporting (no vs yes)	0.282 ± 0.349	0.545	0.305 ± 0.348	0.471
Fibre intake (g/day)	−0.009 ± 0.015	0.381	0.015 ± 0.019	0.502
rs1501299 (GG vs GT + TT)	0.325 ± 0.213	0.140	1.100 ± 0.450	0.014
Interaction [rs1501299 (GG vs GT + TT) × fibre]			−0.049 ± 0.025	0.028
Adjusted R^2 of the model	0.019	0.020	0.026	0.006

However, a dietary pattern characterized by high consumption of whole-grain cereals and low-fat dairy products was modestly positively associated with adiponectin in healthy women [16]. Diets low in glycemic load and high in cereal fibre intake were also reported to be positively associated with circulating adiponectin in diabetics [12]. Cereal fibre may affect adiponectin by promoting the clearance of lipids and thus reduce free fatty acids available for storage in adipose tissue, which act as ligands to activate the upstream regulation of adiponectin expression [5, 6]. Unlike the aforementioned evidence [12, 16], we failed to demonstrate such associations in our study group, but, only under a state of low fibre intake did the GG homozygotes for the rs1501299 SNP present with higher adiponectin concentration when compared to T carriers. This finding may be explained by the fact that non-refined cereal intake consumption was low (servings per day), whereas legumes (servings per day) were the leading source of fibre in our sample. Unlike whole-grain cereals, legumes are rich in soluble fibre and may have a different effect on adiponectin. Moreover, our sample consisted of healthy children in contrast to the study groups of other available studies, which comprised of diabetic or apparently healthy adults.

The frequency of rare T and A alleles of rs1501299 and rs17300539, respectively, was consistent with data reported from HapMap project for European populations [14]. The common G allele of rs1501299 polymorphism has been previously associated with lower adiponectin levels in healthy Caucasians [8] and obese children [1]. However, in a study of non-diabetic Caucasian subjects, the T allele was associated with lower adiponectin [2]. In line with our findings, no significant association between variants of rs1501299 and adiponectin was reported in a case–control study of diabetic subjects [11]. The inconsistency of the aforementioned findings might be attributed to ethnic diversity, age and/or on environmental factors such as diet, which can differ significantly between study populations.

Amongst the strengths of our investigation is the homogeneity of the study group, which included children of Greek origin. At this time, the observational design of our study does not allow the elucidation of mechanisms; further studies are needed to confirm these findings in other population groups, in terms of age, origin and disease state, and propose the underlying pathways.

In conclusion, we demonstrated that the rs1501299 × fibre intake interaction significantly affected serum adiponectin levels in healthy school-aged children of Greek origin: with lower fibre intake, GG, compared to T carriers, might be protected with regard to adiponectin levels and therefore against the risk of obesity and insulin resistance. Our results underline the significance of investigating the effects of gene–diet interactions in studying diet–disease relationships and interventions.

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Conflict of interest statement We declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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