Adiponectin levels do not predict clinical onset of type 1 diabetes in antibody-positive relatives

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Abstract

Aims/Hypothesis Insulin resistance has been proposed as a risk factor for type 1 diabetes. We investigated whether adiponectin, an insulin sensitiser, can serve as an additional predictive marker for type 1 diabetes in first-degree relatives of known patients.

Methods Adiponectin was followed in 211 persistently islet antibody-positive (Ab+) first-degree relatives of type 1 diabetic patients and in 211 age- and sex-matched persis-

tently antibody-negative relatives, and correlated with antibody status, random proinsulin:C-peptide ratio and *HLA-DQ* genotype. During follow-up, 37 Ab+ relatives developed type 1 diabetes.

Results In the group of 422 relatives, baseline adiponectin correlated inversely with age and BMI and was lower in male than in female participants, especially after 15 years of age ($p < 0.001$). There was no correlation with antibody status or later development of diabetes. In 24 Ab+ relatives sampled fasted, adiponectin levels correlated significantly with homeostasis model assessment of insulin sensitivity ($p = 0.006$). In Ab+ relatives ($n = 211$), adiponectin levels could not predict type 1 diabetes nor complement risk assessment based on islet antibodies, *HLA-DQ* genotype and pancreatic hormones in Cox regression analysis.

Conclusions/Interpretation Adiponectin levels do not contribute to the prediction of type 1 diabetes in Ab+ relatives.

I. Truyen and J. De Grijse contributed equally to this study.

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Abbreviations

Ab+	antibody-positive
Ab−	antibody-negative
BDR	Belgian Diabetes Registry
BMI-SDS	BMI standard deviation score
GADA	glutamic acid decarboxylase antibodies
HOMA%S	homeostasis model assessment of insulin sensitivity
IA-2A	insulinoma-associated protein-2 antibodies
IAA	insulin autoantibodies
ICA	islet cell cytoplasmic antibodies
IQR	interquartile range
PI:C	proinsulin:C-peptide ratio

Introduction

Obesity and insulin resistance have been suggested as precipitating clinical onset of type 1 diabetes [1–3]. Since low levels of adiponectin, an insulin-sensitising adipokine, have been associated with obesity, insulin resistance and risk of type 2 diabetes [4, 5], we investigated whether adiponectin can serve as predictive marker for type 1 diabetes in first-degree relatives of known patients. In this group, islet antibodies, especially insulinoma-associated protein-2 antibodies (IA-2A), combined with genetic or beta cell functional markers confer high risk but identify individuals with variable progression rates towards diabetes [6, 7]. We assessed whether adiponectin levels allow identification of relatives at risk of rapid progression to type 1 diabetes.

Methods

Participants and data collection In total, 4,796 siblings and offspring of type 1 diabetic patients, consecutively recruited by the Belgian Diabetes Registry (BDR) between 1 January 1989 and 3 November 2004 according to previously defined criteria [6, 7], were sampled twice or more during a median (interquartile range [IQR]) period of 81 (59–90) months. After obtaining written informed consent, random blood samples were taken and questionnaires were completed at entry and about yearly thereafter.

Adiponectin was determined in available samples of 211 persistently antibody-positive (Ab+) relatives (who tested positive for insulin autoantibodies [IAA], islet cell cytoplasmic antibodies [ICA], glutamic acid decarboxylase antibodies [GADA] and/or IA-2A at inclusion and about 1 year later) and in samples of 211 relatives who remained antibody-negative (Ab-) during ≥ 4 years, individually matched for sex and age with Ab+ relatives. Thirty-seven (18%) of these 211 persistently Ab+ relatives developed type 1 diabetes during follow-up and were reported to the BDR by the diabetologists who made the diagnosis of diabetes. Classification as type 1 diabetes was on the basis of centrally determined autoantibodies. BMI was expressed as a standard deviation score (BMI-SDS) by comparison with an age- and sex-matched reference cohort [6].

The study was conducted in accordance with the guidelines in the Declaration of Helsinki as revised in 2000 (<http://www.wma.net/e/policy/b3.htm>) and approved by the ethics committees of the BDR and the participating university hospitals.

Analytical methods ICA were determined by indirect immunofluorescence and IA-2A, GADA and IAA by

liquid-phase radiobinding assays. In the DASP 2005 programme, sensitivity and specificity were respectively 38 and 98% for IAA, 82 and 93% for GADA and 76 and 98% for IA-2A. *HLA-DQ* polymorphisms, proinsulin and C-peptide were determined as previously described [6, 7]. Random serum adiponectin levels were measured by the Linco ELISA method (Linco Research, St Charles, MO, USA), in which mouse immunoglobulin is added to avoid interference of heterophile antibodies. Interassay CV values were 10.0% ($n=82$) and 7.9% ($n=82$) at the level of 5.5 and 27.3 $\mu\text{g/l}$, respectively. Intra-assay CV values averaged $<5\%$. Changes in adiponectin were studied using delta adiponectin ([adiponectin (mg/l) in first follow-up sample after 1 year]—[adiponectin (mg/l) at entry]). In 24 IA-2A-positive relatives sampled fasted, glucose and insulin levels (BI-insulin IRMA; Schering, Gif-Sur-Yvette, France) were used to determine insulin sensitivity by homeostasis model assessment (HOMA%S) [8]. In 13 relatives who developed diabetes, adiponectin levels were determined after a median (IQR) period of insulin treatment of 10 (6–12) months.

Statistical analysis Statistical differences between groups were assessed by the Mann–Whitney *U* test for continuous variables and by the Wilcoxon signed rank test or Friedman test for paired variables. Spearman's rank correlation was used to investigate the relationship between adiponectin levels and other variables. Stepwise multiple regression analysis was performed to determine the independent contribution of variables on $\log_{10}$ -transformed adiponectin levels. Diabetes-free survival was studied by Kaplan–Meier analysis and a log-rank test [6]. A Cox proportional hazards model, performed by the forward stepwise method, was used to investigate the independent contributions of the strongest risk factors identified by univariate analysis ($p \leq 0.001$) [6]. Statistical tests were performed two-tailed by SPSS for Windows 13.0 (SPSS, Chicago, IL, USA) and considered significant whenever $p < 0.05$ or $p < 0.05/k$ in the case of k comparisons (Bonferroni adjustment) [6].

Results

Baseline adiponectin levels In the group of 422 relatives, initial adiponectin levels were inversely correlated with age (Spearman correlation coefficient [r_s]= -0.318 ; $p < 0.001$). Above the age of 15 years, adiponectin levels were significantly lower in male ($n=95$) than in female ($n=96$) participants (median [IQR]: 10.6 [8.5–12.6] mg/l vs 12.4 [9.4–15.6] mg/l; $p < 0.001$), which was not observed at a younger age (median adiponectin levels [interquartile range]: 12.8 [10.3–16.5] mg/l in 135 boys vs 14.1 [10.2–17.8] mg/l in 96 girls; $p=0.211$). In 329 relatives with

known anthropometric data (see “Methods”) baseline adiponectin levels were inversely correlated with BMI-SDS ($r_s = -0.179$; $p = 0.001$).

Adiponectin levels of Ab+ prediabetic ($n = 37$) and non-diabetic relatives with ($n = 174$) or without ($n = 211$) autoantibodies were largely overlapping (median [IQR] respectively 11.8 [9.4–17.1] mg/l, 12.1 [9.1–15.1] mg/l and 12.5 [9.9–15.8] mg/l; $p = 0.70$) and did not vary according to IA-2A status (not shown). In 24 IA-2A-positive relatives sampled fasted, adiponectin correlated significantly with HOMA%S ($r_s = 0.54$; $p = 0.006$) (Fig. 1).

Multiple regression analysis confirmed that lower adiponectin levels were associated with older age ($p < 0.001$), increased BMI-SDS ($p = 0.039$) and male sex ($p = 0.008$) while immune and genetic predictors of diabetes did not independently correlate with adiponectin (Table 1). Moreover, the inverse relationship of adiponectin with age was also dependent on its interaction with sex ($p < 0.001$) and BMI-SDS ($p = 0.002$) (Table 1).

Changes in adiponectin levels in Ab+ relatives Adiponectin levels decreased two years prior to clinical onset in Ab+ prediabetic relatives ($n = 28$; $p \leq 0.001$) but not in 28 individually age- and sex-matched Ab+ and Ab- non-prediabetic relatives followed over an equally long period (see [Electronic supplementary material](#)).

Predictive value of adiponectin levels in Ab+ relatives In Kaplan–Meier analysis, Ab+ relatives ($n = 211$) showed no differences in progression towards diabetes according to tertiles of baseline adiponectin or changes during follow-up (using delta adiponectin, see “Methods”) (not shown). In univariate Cox regression analysis, ICA positivity, IA-2A positivity, antibody number, *HLA-DQ* risk markers, recurrently high proinsulin:C-peptide ratio (PI:C; greater than 66th percentile of Ab- relatives) and age were significantly associated with progression to diabetes,

Fig. 1 Adiponectin level (mg/l) according to HOMA%S in 24 IA-2A-positive relatives ($r_s = 0.5435$, $p = 0.006$)

Table 1 Multiple linear regression analysis in 329 first-degree relatives (with known BMI-SDS) of type 1 diabetic patients with $\log_{10}$ adiponectin as dependent variable

Independent variables	β coefficient (95% CI)	p value
Model 1 ^a		
Age ^b	−0.006 (−0.008 to −0.004)	<0.001
Sex (1=male, 2=female)	0.050 (0.015 to 0.089)	0.008
BMI-SDS ^c	−0.014 (−0.028 to −0.001)	0.039
Model 2 ^d		
Age ^b	−0.012 (−0.016 to −0.009)	<0.001
Interactions		
Age×sex	0.004 (0.002 to 0.006)	<0.001
Age×BMI-SDS	−0.001 (−0.002 to −0.000)	0.002

Model 2 regression analysis was performed by fitting the significant markers from model 1 and their interactions

^a IAA, ICA, GADA, IA-2A, number of antibodies, *HLA-DQ2*, *HLA-DQ8*, *HLA-DQ2/DQ8*, and high PI:C were also included in model 1 but did not reach statistical significance

^b Variables at study entry

^c BMI-SDS was available for 25 prediabetic, 131 Ab+ and 173 Ab- non-diabetic relatives

^d Sex, BMI-SDS and their interaction were also included in model 2 but did not reach statistical significance

whereas a greater than median decrease in adiponectin and BMI-SDS (β coefficients [95% CI] were 0.68 [0.35–1.31] with $p = 0.248$ and 0.73 [0.52–1.03] with $p = 0.075$, respectively), IAA positivity and GADA positivity were not. The strongest predictors ($p \leq 0.001$ for IA-2A positivity, antibody number, *HLA-DQ* associated risk and high PI:C) were entered in a multivariate Cox regression model confirming the known independent contribution of IA-2A, *HLA-DQ* risk markers and recurrently high PI:C to risk of type 1 diabetes (not shown).

Discussion

Our study demonstrates that low adiponectin levels during the prediabetic phase did not improve prediction of type 1 diabetes in Ab+ relatives. Kaplan–Meier survival analysis did not show a significant association between adiponectin levels and progression to diabetes. The independent contribution of IA-2A, *HLA-DQ* risk markers and high PI:C to diabetes prediction [6, 7] was confirmed by Cox regression analysis but adiponectin (or BMI-SDS) did not improve the predictive model based on these markers.

The presently used ELISA does not provide direct measurement of biologically active high-molecular mass forms of adiponectin that are considered better markers for systemic insulin sensitivity [9]. However, levels of total and high molecular mass adiponectin are strongly correlated [10]. In addition, we found a significant correlation between

circulating adiponectin levels and HOMA%S and confirmed their known relationship with BMI, age and sex [5].

On the basis of the present data, the role of an insulin-resistant state in the progression towards type 1 diabetes could be questioned. Further work on larger series, longer time-frames and other adipokines (e.g. leptin and resistin) is certainly needed before reaching such a conclusion [4, 5].

In conclusion, adiponectin levels decrease shortly before clinical onset of type 1 diabetes but do not complement established markers for prediction of the disease in Ab+ first-degree relatives of known patients.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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