

## Short Report

# An association between Type 2 diabetes and $\alpha_1$ -antitrypsin deficiency

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### Abstract

**Aims**  $\alpha_1$ -Antitrypsin (AAT) is a serine protease inhibitor which recently has been shown to prevent Type 1 diabetes development, to prolong islet allograft survival and to inhibit pancreatic B-cell apoptosis *in vivo*. It has also been reported that Type 1 diabetic patients have significantly lower plasma concentrations of AAT, suggesting the potential role of AAT in the pathogenesis of Type 1 diabetes. We have investigated whether plasma AAT levels are altered in Type 2 diabetes.

**Methods** The study included patients with Type 2 diabetes ( $n = 163$ ) and non-diabetic control subjects matched for age, sex and smoking habits ( $n = 158$ ) derived from the population-based Malmö Diet and Cancer study. Plasma samples were analysed for AAT concentration and phenotype and serum glucose, insulin, C-reactive protein and lipid levels were measured. Glycated haemoglobin was also measured.

**Results** In the diabetic group, the women had higher mean plasma AAT levels than men ( $P < 0.05$ ). The mean plasma AAT levels did not differ between diabetic and control subjects. However, the number of individuals with low AAT levels ( $< 1.0$  mg/ml) was 50% higher in the diabetic group ( $P < 0.05$ ) and the frequency of AAT deficiency genotypes was 50% higher (NS) in diabetic compared with control subjects. In the group of diabetic patients with AAT  $< 1$  mg/ml, AAT directly correlated with systolic blood pressure ( $P = 0.048$ ) and inversely correlated with waist-hip ratio ( $P = 0.031$ ).

**Conclusions** Our results provide evidence that deficiency of AAT may be associated with an increased risk of developing Type 2 diabetes.

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**Keywords**  $\alpha_1$ -antitrypsin, diabetes mellitus, inflammation

**Abbreviations** AAT,  $\alpha_1$ -antitrypsin; BP, blood pressure; CRP, C-reactive protein; HbA<sub>1c</sub>, glycated haemoglobin; HDL, high-density lipoprotein; IEF, isoelectric focusing; LDL, low-density lipoprotein; MDC-CVA, Malmö Diet and Cancer study cardiovascular arm; TG, triglyceride

### Introduction

$\alpha_1$ -Antitrypsin (AAT) is a member of the serine protease inhibitor (serpin) family and is one of the major protective circulating proteins in humans. In addition, AAT regulates other processes, including reactive oxygen species toxicity, cell-mediated immunity/tolerance, neutrophil and endotoxin-mediated inflammation, reproduction, endothelial function

and apoptosis [1–9]. Recently, it has been demonstrated that over-expression of AAT by gene delivery using recombinant adeno-associated virus significantly reduced insulinitis and prevented the development of overt hyperglycaemia in non-obese diabetic (NOD) mice [10,11]. In addition, studies by Lewis and co-workers have shown that administration of clinical-grade human AAT prolongs pancreatic islet allograft survival and exhibits islet-related cytoprotective effects [12]. Although the mechanism by which AAT administration provides these beneficial therapeutic outcomes remains largely unclear, recent studies have demonstrated that treatment with AAT protects pancreatic B-cells against apoptosis through inhibition of caspase-3 activity [7,9]. As  $\alpha_1$ -antitrypsin demonstrates a marked ability to prevent both diabetes formation (*in*

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*vivo*) and B-cell apoptosis (*in vitro*), one could postulate that deficiencies in the quantity of AAT may increase the risk of developing diabetes. In support of this, it has been reported that Type 1 diabetic patients have significantly lower plasma levels of AAT [13]. To date, there is no reported data on the association of plasma AAT levels and Type 2 diabetes. Therefore, we designed this study to assess whether lower plasma levels of AAT are associated with Type 2 diabetes.

## Methods

### Study participants

The study population is derived from the Malmö Diet and Cancer study (MDC) [14]. The protocols were approved by local ethics committees and informed consent was obtained from all subjects. Cardiovascular risk factors were measured in a random subsample of the MDC referred to as the MDC cardiovascular arm (MDC-CVA) ( $n = 6103$ ). In the present study, we included patients from the MDC-CVA previously diagnosed with Type 2 diabetes. Each diabetic patient was assigned a non-diabetic control subject matched for age, sex and smoking habit. In this way, we identified 236 cases and 236 control subjects. Plasma was available for AAT analysis in 163 diabetic and 158 control subjects, forming the final study population.

### Blood chemistry and blood pressure measurements

Serum C-reactive protein (CRP), glucose, total cholesterol, total triglyceride (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol levels and glycated haemoglobin (HbA<sub>1c</sub>) were measured using standard laboratory methods. Detailed descriptions of the methods are found elsewhere [15]. Blood pressure (BP) was measured after 5-min rest in the supine position from the right arm using a mercury sphygmomanometer. Readings were taken on two occasions by trained nurses. The systolic BP was defined by 'phase I' and the diastolic BP defined by 'phase V' Korotkoff sounds.

### Analysis of plasma AAT and determination of AAT phenotype

Plasma AAT levels and AAT phenotypes were analysed by nephelometry and isoelectric focusing (IEF) gel electrophoresis [16] at the Department of Clinical Chemistry in Malmö (Malmö University Hospital, Sweden) according to standardized methods. Genotypes were based on the results from the IEF.

### Statistical analysis

The data were analysed using the SPSS software (version 12.0.1 for Windows; SAS Institute, Cary, NC, USA). All variables were analysed for normal distribution by Kolmogorov–Smirnov test. Group-wise differences were tested by using the unpaired Student's *t*-test and, if normality was rejected, the Mann–Whitney *U*-test was used. Correlation coefficients (*r*) were obtained by using the Spearman rank-order correlation coefficient. Fisher's exact test was used for the calculation of frequencies. Tests showing  $P < 0.05$  were considered to be

significant and data are presented as mean  $\pm$  SD or median (range). Normal plasma AAT levels are between 0.92–1.72 mg/ml, and values  $< 0.9$  mg/ml are considered to represent AAT deficiency [17]. AAT is an acute-phase protein and its concentration rises during inflammation and infections [18]. Therefore, subjects with inherited AAT deficiency may also have higher plasma levels of AAT (up to 1.0 mg/ml). Thus, we divided our study population into subgroups with low ( $< 1.0$  mg/ml) or normal/high ( $\geq 1.0$  mg/ml) AAT levels [17].

## Results

### Clinical and biochemical parameters

Blood pressure and weight parameters were significantly higher in the diabetic compared with the control subjects, whereas smoking habits did not differ between the two groups (Table 1). As expected, the diabetic group had higher plasma glucose ( $P < 0.001$ ), insulin ( $P < 0.001$ ), HbA<sub>1c</sub> ( $P < 0.001$ ), TG ( $P < 0.001$ ), LDL : HDL ratio ( $P < 0.05$ ) and high sensitive (hs)CRP ( $P < 0.001$ ), but lower plasma levels of HDL ( $P < 0.001$ ) compared with control subjects. There was no significant difference in plasma AAT levels between diabetic and control participants (Table 1). In the diabetic group, women had higher plasma AAT levels than men ( $1.33 \pm 0.29$  vs.  $1.24 \pm 0.27$  mg/ml,  $P < 0.05$ ). Next, we selected individuals having plasma levels of AAT  $< 1.0$  mg/ml for phenotyping. Our results revealed that the number of individuals with AAT levels  $< 1.0$  mg/ml was 50% higher in the diabetic group ( $P < 0.05$ ). AAT deficiency genotypes were 50% more common in diabetic compared with control subjects (Table 1). Specifically, we found the MZ and FZ in the control group and the MZ, MS and FM AAT deficiency genotypes in the diabetic group (Table 2).

Plasma AAT levels were not influenced by weight or glycaemic control in the diabetic group (data not shown).

### Relationship between plasma AAT and other variables in the diabetic and control groups

There was a weak, inverse correlation between AAT and TG concentrations ( $r = -0.181$ ,  $P = 0.033$ ,  $n = 139$ ) in diabetic patients with AAT  $\geq 1.0$  mg/ml. In diabetic subjects with AAT  $< 1.0$  mg/ml ( $n = 23$ ), there was a correlation between plasma AAT levels and systolic blood pressure ( $r = 0.417$ ,  $P = 0.048$ ) and an inverse correlation between AAT levels and waist–hip ratio ( $r = -0.450$ ,  $P = 0.031$ ). In the subgroup of control subjects with AAT  $< 1.0$  mg/ml ( $n = 11$ ), we found correlations between AAT and glucose ( $r = 0.711$ ,  $P = 0.014$ ) and LDL : HDL ratio ( $r = 0.673$ ,  $P = 0.033$ ) and TG ( $r = 0.645$ ,  $P = 0.032$ ).

## Discussion

Our study shows, for the first time, that the number of individuals with low AAT levels ( $< 1.0$  mg/ml) is about 50% higher in the diabetic compared with the control group.

**Table 1** Patient demographics and measured plasma markers

Variable	Diabetics ( <i>n</i> = 163)	Controls ( <i>n</i> = 158)
Sex (male/female)	88/75	89/69
Age (mean $\pm$ SD, years)	59.87 $\pm$ 5.99	59.92 $\pm$ 5.97
Smoking (never/current/ex-smoker), <i>n</i>	62/26/65	56/32/66
AAT (mean $\pm$ SD, mg/ml)	1.28 $\pm$ 0.28	1.31 $\pm$ 0.23
AAT deficiency, <i>n</i>	15	7
AAT (< 1 mg/ml), <i>n</i>	23†	11
SBP [median (range), mmHg]	150 (116–210)*	140 (106–190)
DBP [median (range), mmHg]	90 (70–118)*	85 (64–116)
BMI (mean $\pm$ SD, kg/cm <sup>2</sup> )	29.17 $\pm$ 4.87*	25.65 $\pm$ 3.21
Waist (mean $\pm$ SD, cm)	96.52 $\pm$ 13.64*	85.94 $\pm$ 11.53
WHR (mean $\pm$ SD)	0.92 $\pm$ 0.09*	0.87 $\pm$ 0.09
HbA <sub>1c</sub> [median (range), %]	7.30 (5.20–13.70)*	4.80 (3.80–5.80)
Glucose [median (range), mmol/l]	9.80 (6.50–25.60)*	4.80 (3.90–5.60)
Cholesterol [mean $\pm$ SD, mmol/l]	6.18 $\pm$ 1.00	6.29 $\pm$ 1.16
TG [median (range), mmol/l]	1.91 (0.59–7.33)*	1.20 (0.56–5.08)
HDL (mean $\pm$ SD, mmol/l)	1.16 $\pm$ 0.34*	1.32 $\pm$ 0.33
LDL (mean $\pm$ SD, mmol/l)	4.10 $\pm$ 0.93	4.32 $\pm$ 1.04
LDL : HDL ratio [median (range)]	3.80 (1.00–9.50)†	3.40 (1.40–9.30)
Insulin [median (range), mIE/l]	12.00 (2.90–70.00)*	6.00 (2.9–24.00)
hsCRP [median (range), mg/l]	0.27 (0.02–5.13)*	0.13 (0.01–2.22)

\**P* < 0.001.  
†*P* < 0.05.  
AAT,  $\alpha_1$ -antitrypsin; BMI, body mass index; DBP, diastolic blood pressure; HbA<sub>1c</sub>, glycosylated haemoglobin; HDL, high-density lipoprotein; hsCRP, high sensitivity C-reactive protein; LDL, low-density lipoprotein; SBP, systolic blood pressure; TG, triglyceride; WHR, waist-hip ratio.

**Table 2** AAT genotypes in diabetic and non-diabetic control subjects

Diabetic patients		Non-diabetic control subjects	
Genotype	<i>n</i> AAT (mg/ml)*	Genotype	<i>n</i> AAT (mg/ml)
MM	148 1.33 $\pm$ 0.24	MM	151 1.33 $\pm$ 0.20
MZ	10 0.75 $\pm$ 0.09	MZ	6 0.85 $\pm$ 0.07
MS	4 0.88 $\pm$ 0.04	—	—
—	—	FZ	1 0.53
MF	1 0.91	—	—

\*Mean  $\pm$  standard deviation.  
AAT,  $\alpha_1$ -antitrypsin; *n*, number of cases.

Epidemiological studies show that people with Type 2 diabetes have a greater incidence of cardiovascular disease and raised blood pressure than the general population [19,20]. A high waist-hip ratio is a strong predictor of cardiovascular diseases and it is also associated with Type 2 diabetes [21]. It is important to point out that our study group of diabetic patients showed a direct correlation between lower levels of AAT (< 1.0 mg/ml) and systolic blood pressure ( $r = 0.417$ ,  $P = 0.048$ ) and an inverse correlation between low AAT levels and waist-hip ratio ( $r = -0.450$ ,  $P = 0.031$ ). These findings further confirm that changes in plasma AAT levels most likely are associated with Type 2 diabetes.

Previous studies have demonstrated abnormal plasma AAT levels in Type 1 diabetic subjects. For instance, Sandler *et al.* have shown that the plasma AAT concentration, as well as total AAT inhibitory activity, are lower in Type 1 diabetic subjects than in control subjects [13]. In addition, diabetic children have significant lower serum AAT concentration and trypsin inhibitory capacity compared with non-diabetic children [22]. In support of this, it has recently been demonstrated that individuals with uncontrolled Type 1 and Type 2 diabetes mellitus have lower serum trypsin inhibitory capacity compared with control subjects; trypsin inhibitory capacity inversely correlated with the duration of diabetes ( $r = -0.5420$ ,  $P < 0.0001$ ) [23]. In the present study, we were not able to investigate the association between time from diagnosis of Type 2 diabetes and plasma AAT levels. However, further studies will be conducted to test the hypothesis that the true duration of Type 2 diabetes may be linked to the reduction of plasma AAT levels.

Several genetic variants of AAT are associated with low plasma AAT levels. The severe and intermediate AAT deficiency phenotypes mostly result from combinations of S-, Z- and the null alleles. The estimated prevalence of the most important genetic variants of AAT among Caucasians is MM (93/100), MZ (4.6/100), MS (4.7/100), SS (1/1600), SZ (1/750) and ZZ (1/1600). Recent reports suggest the potential existence of at least 116 million carriers of deficiency alleles (Pi MZ and MS) and 3.4 million deficiency allele combinations (Pi SZ, SS and ZZ) worldwide [24]. Severe ZZ AAT deficiency

is convincingly linked to increased susceptibility to the development of chronic obstructive pulmonary disease [25], neonatal hepatitis, liver cirrhosis and hepatocellular carcinoma [26,27]. Individuals heterozygous for the S or Z allele (MZ or MS) have 55–80% of normal plasma AAT levels [28] and, to date, these intermediate AAT deficiency genotypes have not been linked to the development of specific diseases. In this study, we determined AAT alleles in a cohort of 163 patients with Type 2 diabetes and found that 11.5% had heterozygous MZ or MS (9 and 2.5%, respectively) AAT deficiency, whereas only 3.9% of the matched non-diabetic control subjects ( $n = 1558$ ) had MZ AAT. These novel findings suggest a role of AAT in inhibition of pancreatic B-cell apoptosis. Additional clinical studies are required to assessing the genotype of AAT in Type 2 diabetes and compare the degree of B-cell apoptosis in diabetic patients with normal and low levels of AAT.

### Competing interests

Nothing to declare.

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