terol is in line with our hypothesis, based on the observations by Passarelli et al. [2]. Elevated HbA_{1c} may be an indicator of elevated glycation of lipoproteins, which increases cholesterol ester transport away from HDL, thereby causing low HDL-cholesterol levels. Interestingly, our data suggest that, from the viewpoint of maintenance of HDL-cholesterol levels, glycaemic levels have a more important role in NGT and IGT subjects than in Type II diabetic subjects.

In conclusion, we have shown a direct association between HbA_{1c} and HDL-cholesterol in a population-based study. This is in agreement with the in vitro studies of Passarelli et al. [2].

Yours sincerely,
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Diabetologia (1998) 41: 1249–1250

Role of S20G mutation of amylin gene in insulin secretion, insulin sensitivity, and Type II diabetes mellitus in Taiwanese patients

Dear Sir,
Type II (non-insulin-dependent) diabetes mellitus has been known to have a strong genetic component as shown in twin and family studies [1, 2]. Among the candidate genes for Type II diabetes, the mutations found in the insulin gene, insulin receptor gene, insulin receptor substrate gene, glucokinase gene, hepatocyte nuclear factor genes and mitochondrial DNA may only account for 5–10% of patients with Type II diabetes [3]. Recently, a missense mutation of the amylin gene (S20G) has been described and implicated in the pathogenesis of Type II diabetes, especially early-onset diabetes, in Japanese patients [4]. Since the genetic factors contributing to Type II diabetes can vary among different ethnic groups, we searched for the S20G mutation of the amylin gene and investigated its role in diabetes phenotypes and glucose metabolism, including insulin secretion and insulin sensitivity, in a Taiwanese population.

We recruited 99 unrelated normal control subjects, 24 subjects with impaired glucose tolerance (IGT), 122 with Type I diabetes and 182 with Type II diabetes from Chinese people living in Taiwan. The demographic characteristics of the normal control group and the subjects with IGT, Type I and Type II diabetes are listed in Table 1. Except for the subjects with Type I diabetes they were matched for age, sex, and body mass index. The S20G mutation of the amylin gene (Genbank Accession M26650) was detected by polymerase chain reaction and restriction fragment length polymorphism and then confirmed by DNA sequence analyses [4]. Pedigree members of the subjects with this mutation were studied by oral glucose tolerance tests, glucagon stimulation tests, and insulin suppression tests.

The S20G mutation of the amylin gene was found in four patients with normal glucose tolerance, one with IGT, two with Type I diabetes and three with Type II diabetes (Table 1).

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Table 1. Demographic data and the frequencies of S20G mutation of the amylin gene among the subjects studied from Taiwan

	Control	IGT	Type I	Type II
Number	99	24	122	182
Age (years)	55 ± 12	58 ± 10	15 ± 6	54 ± 15
Male : female	60 : 39	18 : 6	54 : 68	85 : 97
Body mass index (kg/m^2)	24.2 ± 3.4	25.1 ± 2.8	19.0 ± 2.8	25.1 ± 3.9
Age at onset (years)	–	–	9 ± 6	48 ± 12
Cases (%) with mutation	4 (4.4%)	1 (4.2%)	2 (1.6%)	3 (1.6%)
95% Confidence interval	0.2–7.9%	3.8–12.2%	–0.6–3.9%	–0.2–3.5%

There was no significant difference in the frequencies of the amylin gene mutation among the subjects with IGT and diabetes as compared with that in the normal control subjects (Table 1, $p > 0.05$). For the normal subjects with the amylin gene mutation, their ages at recruitment were 51, 55, 55 and 68 years, respectively. For the Type I diabetic subjects found to have the amylin gene mutation, the ages at onset were 6 and 3 years, respectively. Human leukocyte antigen DR genotyping showed that they carried DR3/DR4 and DR4/DR4, respectively. Both cases were positive for IA-2 (ICA512) autoantibody but negative for GAD_{65} antibodies. For the Type II diabetic subjects with amylin gene mutation, the ages at onset were 45, 48 and 57 years, respectively. Pedigree studies showed that the S20G mutation of the amylin gene did not co-segregate with the disease, suggesting this mutation was neither sufficient nor necessary for the development of diabetes. We could not find any significant difference in the age at onset, gender, body mass index, waist-hip ratio, systolic and diastolic blood pressures, fasting plasma glucose and insulin levels between the diabetic subjects with and without the amylin gene mutation.

To evaluate the role of the amylin gene mutation on insulin secretion, we studied insulin and C-peptide responses after oral glucose and intravenous glucagon stimulation in the non-diabetic pedigree members. Among them, subjects with muta-

tions had a reduced response of insulin secretion at 30 min and an increase in plasma glucose concentration at 60 min after oral glucose loading as compared with those without mutations. The maximal insulin secretion was delayed in the subjects with mutations (90 min vs 30 min in the subjects without mutations after glucose loading). The significance level of insulin response at 30 min between the two groups was still observed after adjustment for the subject's body mass index ($p < 0.05$) and steady-state plasma glucose ($p < 0.001$) levels, but not observed after adjustment for age ($p > 0.05$). The areas under the curve (AUC) of glucose and insulin responses were no different between the two groups (AUC of glucose: 178.7 ± 33.0 vs 235.9 ± 10.1 mmol/l · min; AUC of insulin: 18353 ± 8051 vs 10643 ± 2479 pmol/l · min). Both basal and stimulated C-peptide levels at 6 min after glucagon injection were not significantly different between the two groups.

To study the effect of the amylin gene mutation on insulin sensitivity, we compared the measured parameters. We found no differences in the body mass index and waist-hip ratio, and the steady-state plasma glucose levels (8.8 ± 1.8 mmol/l vs 10.8 ± 1.6 mmol/l; $p > 0.05$) between the subjects with and without the amylin gene mutation, indicating the absence of any effect of the mutation on insulin resistance. Previous studies of the patients with amylin gene mutations showed an elevated amylin to insulin molar ratio, indicating a relatively high level of amylin concentration to insulin in these subjects [4]. Chronic elevation of the amylin concentration in transgenic animals, however, suggests amylin has no effect on insulin actions [5]. Our studies in humans might agree with these data as we could not show any effect of the amylin gene mutation on insulin sensitivity.

In conclusion, our data indicate the S20G mutation of the amylin gene does not play a role in the pathogenesis of diabetes mellitus. Amylin has been shown to downregulate glucose-induced insulin biosynthesis and secretion by affecting the insulin mRNA and insulin content [6, 7]. We also found a small reduction in early insulin secretion in the non-diabetic subjects carrying amylin gene mutations although this effect was very mild and was not observed after adjustment for age. Whether this effect on beta cells is caused by high levels of S20G-amylin or direct beta-cell toxicity due to increased amyloid fibril formation by S20G-amylin deserves further studies. Our data are not in agreement with the study by Sakagashira et al. [4]. Whether this is due to ethnic differences or genetic heterogeneity is not clear. Both studies, however, conclude that the amylin gene mutation does not seem to play a major role in the pathogenesis of Type II diabetes.

Yours sincerely,
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Diabetologia (1998) 41: 1250–1251

Insulin sensitivity is inversely correlated with plasma cholesteryl ester transfer protein (CETP)

Dear Sir,
Late-onset (≧ 30 yr) Type I (insulin-dependent) diabetes mellitus is rare in Japanese and characterized by relatively high plasma HDL-cholesterol (C) concentrations. We studied 17 (12 women) Type I diabetic patients aged 50.9 ± 11.8 years (mean ± SD) with the following clinical features: age at onset 41.9 ± 9.8 years, episode of keto-acidosis 47.1%, ketosis proneness 94.1%, positive anti-GAD antibody 41.2%, BMI 21.9 ± 2.3, HbA_{1c} 8.7 ± 1.6%, insulin dose 34.8 ± 14.1 U/day, total C 5.5 ± 0.9 mmol/L, HDL-C 2.4 ± 0.6 mmol/L (2 men > 2.1 mmol/L, 8 women > 2.6 mmol/L), and triglyceride 0.87 ± 0.45 mmol/L. When compared with counterparts comprising 29 patients (24 women) who were recruited randomly and had early-onset (< 20 year) Type I diabetes and similar insulin dosage (43.4 ± 13.5 U/day) and glycaemic control (HbA_{1c} 8.6 ± 1.3%), the mean plasma HDL-C concentration was higher in the late-onset group ($p < 0.001$ from 1.6 ± 0.3 mmol/L). To examine the difference in plasma HDL-C concentrations between the two groups, we measured the degree of insulin sensitivity (GIR) using the euglycaemic hyperinsulinaemic clamp method described previously [1]. The mean GIR value was higher in the late-onset Type I diabetes group (5.47 ± 1.49 vs 4.51 ± 1.47 mg · kg^{-1} · min^{-1}, $p < 0.05$). These results were to be expected because plasma HDL-C concentration and insulin resistance are well known to relate inversely both in non-diabetic subjects and those with Type II (non-insulin-dependent) diabetes mellitus. Further to explore a link between insulin sensitivity and plasma HDL-C, plasma concentrations of cholesteryl ester transfer protein (CETP) and HDL subclasses were determined by the enzyme immunoassay and by the ultra-centrifugation method, respectively.

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