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Observation(s)

A novel mutation in islet amyloid polypeptide (IAPP) gene promoter is associated with Type II diabetes mellitus

To the Editor: Islet amyloid polypeptide (IAPP) is thought to be involved in the normal regulation of glucose metabolism because it is synthesized and co-released with insulin from pancreatic islet beta cells [1]. IAPP could have an important physiological role to play because it is the main constituent peptide of islet amyloid deposits, which are a characteristic feature of human Type II (non-insulin-dependent) diabetes mellitus [2]. The mechanisms responsible for the conversion of IAPP to insoluble fibrils, a biochemical characteristic of amyloidogenesis, are not known. An S20G missense mutation in exon 3 of the *IAPP* gene has been reported in 4.1 % of Japanese subjects with Type II diabetes [3]. Studies in COS-1 cells have shown that the S20G mutant amylin displays increased amyloidogenity and increased intracellular cytotoxicity compared with the wild-type amylin [4]. Overexpression of IAPP is also thought to be involved in islet amyloidogenesis and Type II diabetes. We previously demonstrated a higher stimulatory effect of glucose on IAPP than on insulin mRNA levels in human isolated islets [5]. Moreover, the secretory response of both peptides was dissociated, suggesting that overexpression is involved in secretory defects and could contribute to islet IAPP deposition. In addition, some models of transgenic mice overexpressing the human *IAPP* gene are able to form intracellular IAPP fibrils with the subsequent development of hyperglycaemia [6, 7].

We hypothesized that mutations in the *IAPP* promoter region could lead to abnormal regulation or expression of the gene and could, therefore, be associated with Type II diabetes. This study aimed to investigate the presence of mutations in the promoter region of the *IAPP* gene in a Spanish cohort of Type II diabetic patients. The total study population consisted of 316 unrelated Caucasian Spanish subjects. Altogether 186 Type II diabetic patients (98 men and 88 women, mean age 63 ± 10 years, with BMI 28.5 ± 5.3 Kg/m^2, duration of diabetes of 12 ± 9 years) were consecutively selected from our outpatient clinic. These patients fulfilled the World Health Organization criteria for Type II diabetes. The control group consisted of a sample of 130 healthy non-diabetic subjects (65 men and 65 women, mean age 50 ± 14 years, with BMI 26.6 ± 5.3 Kg/m^2), without a family history of diabetes, recruited from among patients' spouses and hospital staff. The study protocol was approved by the Ethics Committee of the Hospital Clinic of Barcelona and all subjects gave their informed consent to their participation in this study.

Mutations were screened by polymerase chain reaction (PCR) and single strand conformation polymorphism analysis (SSCP) and then confirmed by DNA sequence analysis. Four sets of primers were used to amply overlapping regions of the *IAPP* promoter from –571 bp to + 163 bp of the transcription start site. Primers A (-571 to –375): forward 5'-TCCCTGTCATATCTCTGGTA-3', reverse 5'-CCAAGTGACCTCAATGGCTG-3', primers B (-418 to –172): forward 5'-TATTCCTGAAGCTTCATGGG-3', reverse 5'-CGTAGCAAATACACAGTGT-3', primers C (-229 to + 38): forward 5'-ACTTCTGCTGTGTATGACACACCA-3', reverse 5'-GAGTCCAAGCTTGTATCCACTGGA-3', primers D (-85 to + 163): forward 5'-ATGACAGAGGCTCTCTGAGCT-3', reverse 5'-ACACCAAGTGTGCATTTCTCT-3'. We performed PCR in a 50 l volume containing 1.5 mmol/l $MgCl_2$, 0.2 mmol/l dNTPs, 1 μmol/l of each primer, 2.5 units of Taq DNA polymerase and 100 ng of genomic DNA. The PCR conditions were as follows: initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 1 min; annealing at 50–54 °C for 30 sec, and extension at 72 °C for 1 min; and a final extension at 72 °C for 10 min. The amplification products were examined by SSCP analysis and the samples showing an electrophoretic variant pattern were sequenced on a ABI 377 DNA sequencer.

Table 1. Allele and genotype frequencies of the G-to-A mutation in the promoter region of the IAPP gene according to glucose tolerance status

	Allele frequencies		Genotype frequencies	
	G	A	GG	GA
Control subjects	0.930	0.007	0.985	0.015
Type II diabetic patients	0.952	0.048[a]	0.903	0.097[b]

[a] A-allele between Type II diabetic vs control subjects, $p < 0.001$
[b] GA genotype between Type II diabetic vs control subjects, $p < 0.005$

We detected a single heterozygous mutation consisting of a G-to-A substitution at position –132 bp upstream from the transcription start site in 18 patients with Type II diabetes and in 2 control subjects. The frequency of the *GA* genotype was higher in the diabetic population than in control subjects: 9.7 % vs 1.5 %, $p < 0.005$, odds ratio: 6.85, 95 %-Confidence interval: 1.56–30.08 (Table 1). The distribution of genotypes did not differ from Hardy-Weinberg law expectations in the study group as a whole, including patients and control subjects. We did not find any clinical difference between diabetic subjects with and without the promoter variant. The two non-diabetic carriers, aged 26 and 35, did not have clinical antecedents of a family history of diabetes. Unfortunately, they did not consent to an OGTT.

To evaluate the possible contribution of the G-to-A mutation to the development of Type II diabetes, the carriers' families were studied. Altogether 14 first-degree relatives (mean

age 32.4 ± 9.3 years), offsprings from 8 families, were examined by an OGTT and DNA analysis. The presence of the mutation was detected in nine relatives (two homozygotes, seven heterozygotes) while the five remaining were non-carriers. Family members who were either heterozygous or non-carriers of the mutation showed normal glucose tolerance, while both homozygous carriers had altered OGTT. That of the first carrier, a 45-year-old woman, indicated a diagnosis of diabetes mellitus. The second, a 30-year-old woman with a previous history of gestational diabetes, had impaired glucose tolerance.

In this study, we report the first mutation detected in the promoter region of the *IAPP* gene, which is located within an enhancer domain of the *IAPP* promoter [8]. The heterozygous mutation was more prevalent in Type II diabetic patients than in control subjects ($p < 0.005$). In addition, the study in the family cohort identified two homozygous subjects who had disturbed glucose metabolism, pointing towards a potential role of the G-to-A mutation in the pathogenesis of Type II diabetes. Whether this mutation is responsible for amyloid deposition and could thus be used as a marker for beta-cell dysfunction remains to be clarified.

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Observation

The uncoupling protein *3 –55 C→T* variant is not associated with Type II diabetes mellitus in Danish subjects

To the Editor: Recently, several studies have pointed to a role of the uncoupling protein 3 (UCP3) gene in the regulation of whole body energy homeostasis. Transgenic mice over-expressing UCP3 in muscle tissue are lean and resistant to diet induced obesity [1]. However, UCP3 knock-out mice are seemingly normal in phenotype with respect to their body fat percentage, glucose tolerance, and serum lipid profiles, and they are not predisposed for diet-induced weight gain [2]. In humans, linkage was reported between the *UCP3-UCP2* genomic region and resting metabolic rate [3]. Furthermore, studies of human subjects suggest that the *UCP3* promoter *T-allele* positioned at –55 confers decreased risk of Type II (non-insulin-dependent) diabetes mellitus while simultaneously being associated with dyslipidaemia in both sexes [4]. Another study showed association between presence of the *TT-genotype* and increased BMI in a cohort consisting predominantly of morbidly obese women [5]. Also increased waist-to-hip ratio among glucose tolerant and diabetic women was found to be a consequence of carrying the *TT* or the *CT* genotype [6]. Thus, this *UCP3* polymorphism is apparently implicated in several phenotypes associated with Type II diabetes and the metabolic syndrome.

We hypothesized that the T-allele of the *–55 C→T* polymorphism would be less prevalent among Danish Type II diabetic subjects. Therefore, we examined the association between the *UCP3 –55 C→T* polymorphism and Type II diabetes mellitus among 455 patients of Danish ancestry, who were recruited through the outpatient clinic at Steno Diabetes Center, Copenhagen. Type II diabetes was diagnosed by the 1985 WHO criteria. The Type II diabetic patients (191 women, 264 men) had an average age of 61 ± 11 years (means ± SD), diabetes was diagnosed at 55 ± 11 years of age, a fasting plasma glucose of 8.2 ± 2.9 mmol/l, and an HbA_{1c} of 8.0 ± 1.6 %. All were negative for GAD65 antibodies. The allele and genotype frequency of the *–55 C→T* variant in diabetic patients was compared with the frequencies in two population-based samples of 301 sixty-year-old and 220 middle-aged glucose tolerant Danish control subjects. All control subjects were living in the same area of Copenhagen as the Type II diabetic patients, and their phenotypes have been reported [7]. The study protocols were approved by the ethics committee of Copenhagen and were in accordance with the declaration of Helsinki. Biochemical variables were analysed using Steno Diabetes Center routine methods and genotyping was done as described previously [7]. Differences in genotype frequencies between diabetic and glucose tolerant control subjects were tested using a chi-square test. Phenotypic differences between *–55 C→T* genotypes among the diabetic patients were assessed by analysis of cova-