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Short communication

S20G mutation of the amylin gene is associated with Type II diabetes in Japanese

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Abstract

Aims/hypothesis. Amylin is a unique constituent peptide of the amyloid deposits found in pancreatic islets in many patients with Type II (non-insulin-dependent) diabetes mellitus. A previous study suggested that a missense mutation at amino acid 20 (AGC^{Ser} to GGC^{Gly}), the *S20G* mutation, could play a role in the pathogenesis of early-onset Type II diabetes in Japanese people. In order to determine the association between the *S20G* mutation and Type II diabetes in the Japanese population, we did a large scale screening for the mutation in randomly selected Type II diabetic patients and non-diabetic control subjects.
Methods. We examined 1538 unrelated patients with Type II diabetes and 1108 non-diabetic control subjects recruited from 9 university hospitals and their affiliated hospitals in 7 prefectures in Japan. The presence or absence of the *S20G* mutation of the amylin gene was assessed by direct DNA sequencing or *Msp*I RFLP analysis of the amplified polymerase chain reaction products of exon 3.
Results. The *S20G* mutation was found in 40 (2.6 %) and 9 (0.8 %) of the subjects in the Type II diabetic and the non-diabetic control groups, respectively, all present in the heterozygous state. The frequency of individuals with the *S20G* mutation is different between the two groups ($p = 0.0007$).
Conclusion/interpretation. These data suggest that the *S20G* mutation in the amylin gene is associated with increased risk of development of Type II diabetes in Japanese. [Diabetologia (2001) 44: 906–909]

Keywords Amylin, gene, Type II diabetes, association study, genetics.

Islet amyloid polypeptide, amylin, is a unique constituent peptide of amyloid deposits in pancreatic islets that are found in many Type II diabetic (non-insulin-dependent) patients and in human insulinoma [1–3]. Amylin is derived from an 89-amino acid precursor and co-secreted with insulin and C-peptide in response to nutrient stimuli. In an early analysis of the amylin gene in a small number of Type II diabetic patients including African-Americans and Caucasians, no amylin mutation was found [4]. Investigators, however, recently found the *S20G* missense mutation in 12 (4.1 %) of 294 Japanese Type II diabetic patients and none in 187 non-diabetic control subjects, suggesting an association of the *S20G* mutation with Type II diabetes in the Japanese [5]. Later, two independent studies claimed to show the *S20G* mutation to be more frequent in Type II diabetic subjects than in control subjects in the Japanese but the statistically significant differences in frequency between Type II diabetic patients and control subjects are not clear in either study, possibly due to the small sample size used [6, 7]. Two similar investigations have been made in Asia. An association between the *S20G* mutation and Type II diabetes was found in a Chinese

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Abbreviations: S20G, Serine to Glycine at amino acid 20

cohort, but no significant difference in frequency of the *S20G* mutation between subjects with impaired glucose tolerance or diabetes and control subjects matched for age in a Taiwanese cohort was reported [8]. No mutation was found in either diabetic patients or normal subjects in three studies of Greek, Dutch [9], and North European populations [10]. Accordingly, the importance of the *S20G* mutation as a genetic factor in Type II diabetes is still controversial. In addition to differences in genetic background of various populations, sample size and criteria for non-diabetic control subjects are important factors in case-control studies. We performed a large-scale screening for the *S20G* mutation of the amylin gene as a multi-centre collaboration in Japan. By using strict criteria for non-diabetic control subjects, we were able to determine the association between the *S20G* mutation and Type II diabetes in Japanese.

Subjects and methods

Subjects and mutation analysis. We studied 1,538 unrelated Japanese Type II diabetic patients recruited from 9 university hospitals and affiliated hospitals located in 7 prefectures in Japan. Type II diabetes was diagnosed using the WHO criteria. We also studied 1,108 non-diabetic control subjects, using the following criteria: 60 or more years of age, no past history of diagnosis of diabetes, HbA_{1c} less than 5.6% and no diabetes in family members or three degree relatives. Genetic analysis of human subjects was approved by the ethics committee at each university. Appropriate informed consent was obtained from all the subjects examined, including the volunteer control subjects.

To assess the presence of the *S20G* missense mutation in the amylin gene, exon 3 was amplified by the polymerase chain reaction and the product was tested by direct DNA sequencing or RFLP using restriction enzyme *Msp*I as described [5].

Statistical analyses. The differences in distribution of genotype frequencies between Type II diabetic patients and control subjects were assessed by the chi-square test (Fisher's exact test) from $2 \cdot 2$ contingency tables. The odds ratio for the frequency of the mutant allele in the diabetic group in relation to the control group was obtained. All data are presented as means ± SD. A p value of less than 0.05 was considered statistically significant. All tests and calculations were done with the statistical software package SAS 6.12 (SAS Institute, Cary, N.C, USA).

Results

Clinical data of the Type II diabetic patients and non-diabetic control subjects are summarized in Table 1. The *S20G* mutation was found in 40 (2.6%) and 9 (0.8%) subjects in the diabetic and control groups, respectively, all present in the heterozygous state, showing a significant difference in frequency of individuals with the mutation allele between the two groups (Fisher's exact test, $p = 0.0007$). The allele frequencies of the Type II diabetic patients and non-diabetic control subjects are 1.30% and 0.41%, respectively. The odds ratio for the frequency of the mutant allele in the diabetic group in relation to the control group (the 95%-confidence interval) is 3.23 (1.63–6.42) (Table 2), clearly indicating that the mutation is associated with Type II diabetes in the Japanese cohort.

Table 1. Clinical data from Type II diabetic patients and non-diabetic control subjects[a]

	Type II diabetic patients	Control subjects
Men/women	907/631 (1538)	535/573(1108)
Age	60.6 ± 11.5 (1538)	72.1 ± 8.5 (1108)
Age at diagnosis	47.1 ± 11.9 (1356)	
BMI of women	23.72 ± 3.87 (610)	22.09 ± 3.38 (559)
BMI of men	23.31 ± 3.07 (900)	22.02 ± 3.11 (528)
HbA_{1c} (%)	7.59 ± 1.62 (1514)	5.07 ± 0.36 (1108)
Current therapy		
Insulin (%)	25.8 (348/1347)	
Oral hypoglycemic agents (%)	37.7 (508/1347)	
Diet (%)	36.5 (491/1347)	

[a] Data are expressed as means ± SD; parentheses indicate number of the subjects
BMI, body mass index

Table 2. Prevalence of amylin gene *S20G* mutation in Type II diabetic patients and non-diabetic control subjects [W, wild (AGC^{Ser}), M, mutant (GGC^{Gly})][a]

Genotype	Type II diabetic patients	Control subjects
Number of the subjects	1538	1108
Genotype		
W/W	1498	1099
W/M	40	9
M/M	0	0
Allele frequency (%)	1.30 ± 0.40[a]	0.41 ± 0.26

[a] Data are expressed as means ± SD by assuming binominal distribution. Genotype W/M contribution to chi-square = 11.3 ($p = 0.0007$ by Fisher's exact test). The odds ratio (its 95%-confidence interval) for the frequency of the mutant allele in the diabetic group in relation to the control group is 3.23 (1.63–6.42)

Discussion

It is known that most Type II diabetes in Asia is characterized by defects in insulin secretion [11], rather than insulin resistance, emphasized as the primary defect in Type II diabetes in Western countries [12]. Some of the genes of susceptibility to Type II diabetes in Japanese, accordingly, should be related to pancreatic beta-cell function. Because amylin is a constituent peptide of the amyloid deposits found in the pancreatic islets of many Type II diabetic patients [1–3], mutation of the amylin gene might be associat-

Table 3. Comparison of prevalence of amylin gene *S20G* mutation among different populations[a]

Populations	Prevalence of *S20G* mutation		Reference
	Type II diabetic patients	Control subjects	
Japanese	40/1538 (2.6%)*	9/1108 (0.8%)	This study
	12/294 (4.1%)	0/187 (0%)	(5)
	4/86 (4.7%)	3/184 (1.6%)	(6)
	3/308 (1.0%)	1/149 (0.7%)	(7)
Chinese	17/609 (2.8%)**	1/216 (0.5%)	–
Taiwanese	3/182 (1.6%)	4/99 (4.0%)	(8)
African-American	0/13 (0%)	ND	(4)
Greek	0/15 (0%)	ND	–
Dutch	0/141 (0%)	ND	(9)
North European	0/96 (0%)	0/64 (0%)	(10)

* $p < 0.001$, ** $p < 0.05$ ND, not determined

[a] Frequencies of the mutation in Chinese and Greek subjects were obtained from supplemental issues of *Diabetologia* (41: A110, 1998 and 40: A167, 1997, respectively)

ed with susceptibility to Type II diabetes in Japanese. The present investigation using a large sample population reveals an association between the *S20G* mutation in the amylin gene and Type II diabetes in Japanese, and indicates that the mutation predisposes to Type II diabetes with a relative risk of 3. 23.

Although the residue of Ser^{20} does not locate in the critical "amyloidogenic region" defined in the human amylin protein, the *S20G* mutation might promote amyloid deposits in the pancreatic islets that reduce insulin-secreting cell mass and insulin secretion [13]. In addition, the administration of amylin delays gastric emptying, the effect of which is now being examined in a clinical trial using an analogue to ascertain improvement of postprandial hyperglycaemia in diabetic patients [14]. It is possible that the *S20G* mutant amylin decreases these beneficial effects to contribute to increased risk.

An early study of patients with Type II diabetes including African-Americans and Caucasians failed to identify any mutations in the gene [4]. Recent studies also found no mutations in the gene in Greek, Dutch [9], and North European subjects [10]. On the other hand, Chinese and Taiwanese groups have reported the presence of the mutation at a frequency similar to the Japanese cohorts [6, 7]. However, the small sample sizes in these studies preclude conclusions on the importance of the S20G mutation in Type II diabetes. The known prevalence of the *S20G* mutation in the amylin gene among populations is summarized in Table 3.

The present study shows that both large scale and strict criteria for non-diabetic control subjects are critical in a case-control study of genetic susceptibility for Type II diabetes. Our criteria for non-diabetic control subjects, i.e. 60 or more years in age, no past history of diagnosis of diabetes, an HbA_{1c} level under 5.6%, and no family history of diabetes within three degrees of relatives are more likely to exclude subjects who will later develop diabetes. Because Type II diabetes often develops in middle-age or later, the age of non-diabetic control subjects is especially important and older subjects are more appropriate control subjects for studying genetic susceptibility.

In conclusion, we have confirmed an association between the *S20G* mutation of the amylin gene and Type II diabetes in Japanese that should provide insight into the diverse genetic background of diabetes among populations. We also demonstrate that the criteria for non-diabetic control subjects is critical in case-control studies of gene susceptibility for Type II diabetes.

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