No Association Between the Gly^{971}Arg Variant of the Insulin Receptor Substrate 1 Gene and NIDDM in the Taiwanese Population

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OBJECTIVE — To study the role of the Gly^{971}Arg variant of the insulin receptor substrate 1 (IRS-1) gene in the development of NIDDM in the Chinese population living in Taiwan.

RESEARCH DESIGN AND METHODS — A total of 82 unrelated normal control subjects, 89 subjects with NIDDM, and 23 multiplex families were recruited in Taiwan. All of them were Han Chinese. Pedigree members without a history of diabetes were studied by the standard 75-g oral glucose tolerance test. Detection of the Gly^{971}Arg variant of the IRS-1 gene was performed by polymerase chain reaction and restriction fragment-length polymorphism analysis.

RESULTS — The frequency of Gly^{971}Arg variant of the IRS-1 gene in the normal population was 1.2%, which was lower than frequencies reported in white populations. The prevalence of the Gly^{971}Arg variant was not significantly increased in both the nonselected NIDDM population (1.1%) and the probands of the multiplex families (4.3%). More importantly, the Gly^{971}Arg variant of the IRS-1 gene did not cosegregate with BMI and NIDDM in these families.

CONCLUSIONS — The Gly^{971}Arg variant of the IRS-1 gene is an infrequent normal allele among Taiwanese. This variant is neither associated nor cosegregated with NIDDM in the Taiwanese population and families. Gly^{971}Arg of IRS-1 gene does not play an important role in the development of NIDDM in this population.

NIDDM has been known to have a strong genetic component as demonstrated in twin and family studies (1–4). Among the candidate genes, mutations in the insulin gene, insulin receptor gene, glucokinase gene, and mitochondrial DNA are associated with only 5% of the cases (5–8). Despite the intensive search for the genes responsible for NIDDM, the genetic factor for the majority of NIDDM is still a geneticist's nightmare (9,10) due to the variable age of onset, excess mortality, unknown mode of inheritance, and genetic heterogeneity (9,10).

After insulin binds to the surface receptor, the activated receptor tyrosine kinase begins to phosphorylate receptor protein itself and cause the tyrosine phosphorylation of an intracellular substrate, which is named insulin receptor substrate 1 (IRS-1) (11–13). We have previously shown that IRS-1 is crucial in mediating insulin and IGF-1 functions (14) via association with several SH2-containing proteins, such as the p85 subunit of the phosphatidylinositol 3-kinase and grb2 (15,16). Although IRS-1 plays such an important role in insulin signaling immediately after insulin binds to the cells, the role of the IRS-1 gene in the development of diabetes is not well established. In white populations, an increased frequency of codon 971 variation of the IRS-1 gene was found in NIDDM patients compared with the control subjects, suggesting its potential role in the pathogenesis of NIDDM (17–20). However, population stratification and admixture may cause the disease association with certain alleles in the absence of linkage. Indeed, variations of the codons 512 and 971 of IRS-1 do not cosegregate with NIDDM in French families (18).

In the Taiwanese population, the prevalence of NIDDM is relatively low compared with those in Western countries (21). Epidemiological studies in Taiwan revealed that the prevalence and incidence of NIDDM increase as body build becomes heavier, although the prevalence of obesity is very low in this population compared with white populations (22). The genetic factors contributing to NIDDM may be different among different ethnic groups. Therefore, we present the first report of the association and linkage data of the codon 971 variation of the IRS-1 gene in control, NIDDM, and multiplex NIDDM pedigrees from the Taiwanese population.

RESEARCH DESIGN AND METHODS

— The study subjects included 82 unrelated normal control subjects, 89 unrelated NIDDM patients, and 23 multiplex NIDDM pedigrees recruited from the Han Chinese living in Taiwan. The normal control subjects were recruited from those who attended a general health checkup in the National Taiwan University Hospital. The inclusion criteria for patients with NIDDM were as follows: *1*) a blood glucose level that met the World Health Organization (WHO) criteria (23) or use of sulfonylurea agents or

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Received for publication 12 September 1995 and accepted in revised form 21 November 1995.

IRS-1, insulin receptor substrate 1; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; WHO, World Health Organization.

Table 1—*Demographic data of the subjects in this study*

	Control subjects	NIDDM patients	Unrelated probands in multiplex families
n	82	89	23
Age (years)	56 ± 10	60 ± 14	58 ± 15
BMI (kg/m^2)	24.7 ± 3.5	25.1 ± 3.1	24.9 ± 3.6
Age of onset of diabetes (years)	—	51 ± 11	47 ± 10

Data are means ± SE.

insulin for diabetes control; 2) no insulin therapy needed within 1 year of diagnosis; and 3) absence of a history of diabetic ketoacidosis. The status of glucose tolerance of the subjects without known diabetes was assessed by a 75-g oral glucose tolerance test after an overnight fast according to the WHO criteria. This study was approved by the institutional human study committee of the National Taiwan University Hospital, and informed consents were obtained from the participants.

Peripheral blood leukocyte DNA was extracted. A missense Gly-to-Arg change of codon 971, numbered according to our previous report (24), of the IRS-1 gene was detected by polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) (20). Briefly, 0.25 μg genomic DNA was amplified with a forward primer (3339 5′ CTT CTG TCA GGT GTC CAT CC 3′ 3358) and a reverse primer (5′ TGG CGA GGT GTC CAC GTA GC 3′ 3582) (20). An amplification without target DNA was always included to check for contamination. The PCR products were digested with *Bst* NI and then subjected to 8% PAGE. A digestion with λ-DNA was included to detect digestion failure. The wild-type gene gave three bands sized 23, 81, and 158 bp while the heterozygous Gly^{971}Arg variant gave five bands sized 23, 81, 158, 107, and 51 bp after *Bst* NI digestion. The nucleotide variation was confirmed by direct DNA sequencing analysis of the PCR products with a DNA sequencing kit (AmpliCycle, Perkin-Elmer/Cetus, Norwalk, CT).

Statistical analysis

Significance of the difference of gene frequency between groups was analyzed by the χ^2 test. $P < 0.05$ was considered statistically significant.

RESULTS— The demographic characteristics of the normal control subjects and the patients with NIDDM were not different in this study (Table 1). In our population, the Gly^{971}Arg variant of the IRS-1 gene was found in 1 of 82 (1.2%) control subjects and 1 of 89 (1.1%) subjects with NIDDM. There was no difference between the normal and NIDDM subjects. Among the probands of the multiplex NIDDM pedigrees, 1 of 23 (4.3%) was found to have the Gly^{971}Arg variant of the IRS-1 gene. As shown in Table 2, there was no significant difference in the prevalence of the Gly^{971}Arg variant among the normal control subjects of different ethnic origin, although the frequency in Taiwan was lower. In the combined data of our unrelated subjects from general NIDDM and multiplex families, the frequency of Gly^{971}Arg variant was significantly lower than in the combined data from white populations (20). The nucleotide change of codon 971 was confirmed by direct DNA sequencing analysis (data not shown).

Among the 23 Taiwanese multiplex NIDDM families, only pedigree 1 was identified to have the Gly^{971}Arg variant (Fig. 1). The clinical manifestation and demographic data of members of this pedigree are listed in Table 3. The distribution of BMI and status of glucose tolerance was not different in patients with the wild-type and Gly^{971}Arg variants of the IRS-1 gene. As shown in Fig. 1, the Gly^{971}Arg variant did not cosegregate with NIDDM and BMI in this pedigree.

CONCLUSIONS— The role of IRS-1 in insulin signaling and its biofunction is well established (11–16). However, the association between certain amino acid changes in the IRS-1 molecules and NIDDM has been conflicting as reported from white populations (17–20). In this study, we present the first report of association of codon 971 variant of the IRS-1 (IRS-1^{971}) gene and NIDDM in the Chinese population and the multiplex families living in Taiwan. We showed that the frequency of the Gly^{971}Arg variant of the IRS-1 gene in Taiwan is relatively low compared with those reported from other ethnic groups (20). There were no significant differences for this polymorphism in the control and diabetic populations. In the pedigree affected, the Gly^{971}Arg variant of the IRS-1 gene did not cosegregate with NIDDM.

The etiology of NIDDM is complex, and clinical heterogeneity hampers the search for diabetogenic factors in each patient. This study illustrates the difficulty in finding a gene for a disease that is characterized by a late age of onset and premature mortality. By simple inspection of our pedigree, the Gly^{971}Arg variant of IRS-1 gene is neither necessary nor sufficient for the development of NIDDM. Moreover, this IRS-1 variant did not seem to be correlated with BMI. The frequency of this variant in our general population is very low compared with figures of ~5–10% (average 6.5%) in the reported series

Table 2—*Frequency of IRS-1^{971} variant among different ethnic groups*

Ethnic group	Frequency of Gly^{971}Arg variant of the IRS-1 gene: Control subjects	NIDDM patients
Present study	1/82 (1.2)	1/89 (1.1)
		1/23 (4.3)*
Combined		2/112 (1.8)†
Danish	3/76 (3.9)	10/86 (11.6)
French	9/130 (6.9)	26/233 (11.2)
Finnish	3/42 (7.0)	3/40 (7.5)
South India	5/95 (5.3)	13/126 (10.3)
Combined	29/447 (6.5)	63/597 (10.6)

Data are *n* (%). *Frequency of the unrelated probands in the multiplex NIDDM families in Taiwan. †$P < 0.01$ vs. combined data from white populations.

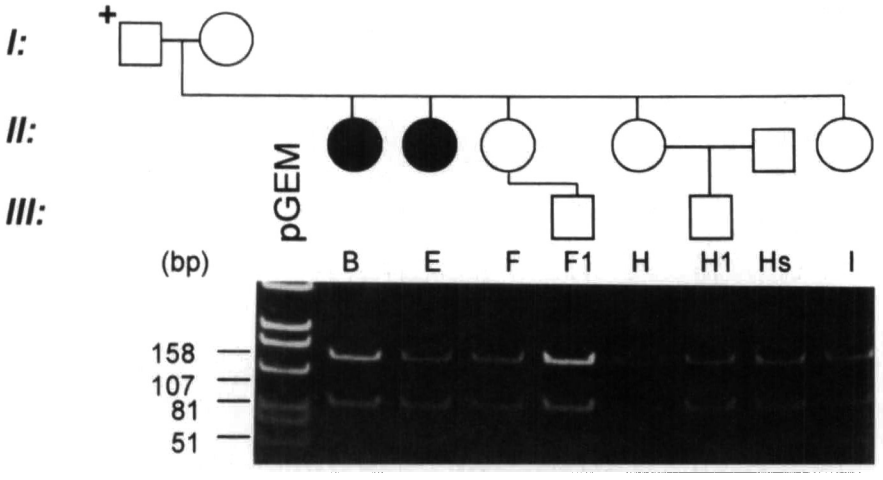

Figure 1—*Pedigree with Gly^{971}Arg variant of the IRS-1 gene. The pedigree tree of multiplex NIDDM family 1 with the Gly^{971}Arg variant of the IRS-1 gene is shown in the upper panel. ●, diabetes. The PCR-RFLP picture for the Gly^{971}Arg variant of the IRS-1 gene is shown in the lower panel. DNA was amplified and digested with* Bst *NI restriction endonuclease. The DNA fragments were then separated by PAGE and stained by ethidium bromide. pGEM DNA marker (Promega Biotec, Madison, WI) is shown on the left. The appearance of 107- and 51-bp fragments denotes Gly^{971}Arg variant of the IRS-1 gene.*

(20), suggesting that the Gly^{971}Arg variant of the IRS-1 gene does not have a major role in the development of NIDDM in this population. Although none of the individual studies showed a statistically significant increase of the IRS-1^{971} variant in NIDDM patients, the result became significant when data from the white populations in Finland, Denmark, France, and South India were combined after a homogeneity test (20). In white NIDDM patients, the average frequency of the IRS-1^{971} variant was 10.6% (vs. 6.5% in the control group, $P < 0.02$). However, the positive association between a gene and disease still might come from population stratification and admixture. Therefore, a more sensitive test for the presence of linkage, such as haplotype relative risk (25) or a transmission/disequilibrium test (26), may be necessary to provide evidence of linkage. For the limited data from the Oriental populations, our results await further confirmation.

In conclusion, the Gly^{971}Arg variant of the IRS-1 gene is an infrequent normal allele in the Taiwanese population. This variant is neither associated nor cosegregated with NIDDM in the Taiwanese. Further demonstration of the biological function of IRS-1^{971} variant in insulin signaling pathways will be needed to elucidate its potential role in the development of NIDDM.

Table 3—*Clinical characteristics of the pedigree subjects with the Gly^{971}Arg variant of the IRS-1 gene*

Case	Age (years)	BMI (kg/m^2)	Status	Age of onset of diabetes (years)	Treatment
IRS-1^{971} variant (+)					
IIE	46	30.6	NIDDM	45	OHAs
IIH	36	25.6	NGT	—	—
IIH1	12	27.7	NGT	—	—
IRS-1^{971} variant (−)					
IIB	53	29.2	NIDDM	51	OHAs
IIF	42	29.3	NGT	—	—
III	32	25.4	NGT	—	—
IIIF1	10	20.4	NGT	—	—

NGT, normal glucose tolerance; OHAs, oral hypoglycemic agents.

Acknowledgments— This work was supported by Grant IBMS-CRC84-T12 from the Institute of Biomedical Science of the Republic of China.

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