行政院國家科學委員會補助專題研究計畫成果報告

分析在NIH3T3-L1脂肪細胞胰島素抗阻性相關基因-從基礎到臨床之研究

計畫類別：■個別型計畫　　□整合型計畫
計畫編號：NSC 90－2314－B－002－145
執行期間：90年8月1日至91年7月31日
計畫主持人：莊立民
共同主持人：
計畫參與人員：張恬君、蔡明翰、江怡德、李伯亨

本成果報告包括以下應繳交之附件：

□赴國外出差或研習心得報告一份
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執行單位：臺大醫學院、內科

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行政院國家科學委員會專題研究計畫成果報告

中文計畫名稱：分析在 NIH3T3-L1 脂肪細胞胰島素抗阻性相關基因-從基礎到臨床之研究
英文計畫名稱：Characterization of the gene involved in insulin resistance in the NIH3T3-L1 adipocytes- from basic to clinical studies
計畫編號：NSC 90-2314-B-002-145
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主持人：莊立民
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一、中文摘要

在不同種族 *ADRB2* 基因之變異，與肥胖和第二型糖尿病之關係並未確定，本研究針對國人之 *ADRB2* 基因多型性變異與第二型糖尿病之發生及發病年齡之關係加以分析。本研究共分析兩個胺基酸之多型性變異，並採用個案控制之設計，分別找出兩群有相同性別、年齡與身體質量指數之正常人（130 人）及第二型糖尿病病人（130 人），結果發現 Arg16Gly 之變異頻率再兩組間有差異(P = 0.039)，在多變項回歸分析亦證明 Arg16Gly 之變異與與第二型糖尿病之發生有獨立之影響(P = 0.021)，且與發病年齡有關(P = 0.017)。至於另一個基因之變異，Gln27Glu 多型性變異則與第二型糖尿病之發生無關。因此吾等作出結論，*ADRB2* 基因型若為 Arg16 之同型接合子，其發病危險性增加 1.87 倍(95% 信心區間為 1.34-2.40)，且發病年齡也較早。

關鍵詞：*ADRB2*、基因多型性變異、第二型糖尿病、台灣人

Abstract

OBJECTIVE: The significance of the association of amino terminal polymorphisms in beta2-adrenoreceptor (ADRB2) with obesity and type 2 diabetes is controversial and differs among ethnic groups. In this study, the association of ADRB2 with risk and age of onset of type 2 diabetes has been examined in a Taiwanese population. DESIGN: The study design is a case-control study to investigate the impact of the two amino acid polymorphisms in ADRB2.

PATIENTS AND MEASUREMENTS: This study includes 130 patients with type 2 diabetes [female : male = 1 : 1, age: 52.4 +/- 10.0 years; body mass index (BMI): 24.2 +/- 2.9 kg/m^2; mean +/- SD] and 130 controlled subjects matched for gender, age and BMI with normal glucose tolerance (female : male = 1 : 1, age: 51.7 +/- 10.6 years; BMI: 23.9 +/- 2.7 kg/m^2). The Arg16Gly and Gln27Glu polymorphisms of ADRB2 were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assays. The genotypic and allelic frequencies between two groups were compared and the relationship between the genotypes and clinical phenotypes was examined.

RESULTS: A difference in genotypic frequency in the Arg16Gly polymorphism was noted between groups in this gender-, age- and BMI-matched case-control study (P = 0.039). Multivariate regression analysis revealed that the Arg16Gly polymorphism was the only independent factor for development of type 2 diabetes (P = 0.021). In addition, we utilized the log-rank test to compare the differences in age of onset between wild-type and nonwild-type polymorphisms. The Arg16Gly polymorphism was independently associated with age of onset in type 2 diabetes (P = 0.017). There was no difference in the

Gln27Glu polymorphism between diabetic and control groups in this study.

CONCLUSIONS: In a Taiwanese population, homozygosity of Arg16 in the ADRB2 gene was associated with a higher frequency (odds ratio 1.87, 95% confidence interval 1.34-2.40) for development of type 2 diabetes. Moreover, this polymorphism was also associated with an earlier onset of type 2 diabetes. However, the Glu27Gln polymorphism had no impact on either BMI or type 2 diabetes in a Taiwanese population.

Keywords:
β2-adrenoreceptor, *ADRB2*, genetic polymorphism, type 2 diabetes, Taiwanese

二、緣由與目的

Obesity is one of the major health issues in developed countries. It is not only associated with increased mortality but is also associated with an increased frequency of type 2 diabetes, gallbladder disease, coronary heart disease, hyperlipidaemia, hypertension and osteoarthritis. It is generally accepted that obesity results from a positive balance in energy homeostasis governed by complex interactions among many genetic and environmental factors. A key process in the energy balance is the mobilization of lipids through lipolysis in adipocytes. Catecholamines play a central role in the regulation of energy expenditure, in part by stimulating lipid metabolism through lipolysis in fat cells. All three known subtypes of β-adrenergic receptors (*ADRB*) promote lipolysis in human adipose tissue *in vivo*, although the three *ADRB* subtypes are differently expressed among tissues. *ADRB2* and *ADRB3* are expressed in abdominal subcutaneous adipose tissue but *ADRB2* seems to be of greater importance than *ADRB3* for the mobilization of lipids. It has also been shown that the sympathetically mediated thermogenic response to stimuli is related to the stimulation of both *ADRB1* and *ADRB2*, but not *ADRB3*).

Although several polymorphisms have been found in the coding region of the *ADRB2* gene in humans, only three of them affect amino acid coding. These include the rare Thr164Ile variant and two common Arg16Gly and Gln27Glu variants. In Swedish women, Glu27 was found to be a risk factor for obesity. Subjects homozygous for Glu27 had higher indices of obesity, higher body fat, larger fat cell volume and higher fasting insulin concentrations when compared to those with the Gln27 allele. The Glu27 allele was identified as a risk factor for both obesity and diabetes in a Japanese population. In contrast, the Gln27 allele is not only a risk factor for diabetes but is also associated with insulin resistance in a family-based population study of another Swedish population. The discrepancy in the reported incidence of the Gln27Glu substitution may be due to differing ethnicity and gender in the various studies. However, no association was found between the Arg16Gly polymorphism and obesity in Swedish women and in a Japanese population. However, the frequency of Gly16 homozygotes was lower in obese Japanese women than in nonobese Japanese women. This dichotomy may also be attributable to ethnicity and gender. Functional studies revealed that the Arg16Gly polymorphism was associated with altered *ADRB2* function, with Gly16 carriers showing a fivefold increase in agonist sensitivity without any change in *ADRB2* expression. A separate study reported that an *ADRB2* with the Gln27Glu substitution expressed in Chinese hamster fibroblasts exhibited altered protein conformation and was completely resistant to agonist-induced receptor down-regulation.

三、結果與討論

Genotype and allele frequency of the ADRB2 gene polymorphism on codon 16 and codon 27

The observed genotypic frequencies of the Arg16Gly and Gln27Glu polymorphisms were in compliance with the Hardy-Weinberg equilibrium. The arginine and glycine allele frequencies of codon 16 in nondiabetic subjects were 52·7% (137/260) and 47·3% (123/260), respectively. The allelic frequencies of arginine and glycine in diabetic patients were 58·5% (152/260) and 41·5% (108/260), respectively. The glutamine and glutamate allele frequencies of codon 27 in nondiabetic subjects were 92·3% (240/260) and 7·7% (20/260), respectively. The allelic frequencies of glutamine and glutamate in diabetic patients were 91·9% (239/260) and 8·1% (21/260), respectively. The genotypic distribution of codon 16 between the two groups was also statistically significant. The wild-type allele was associated with a higher frequency of diabetes in this population, with an odds ratio (OR) of 1·87 [95% confidence interval (CI) 1·34-2·40]. In contrast, no difference was noted in the genotypic distribution and allelic frequencies of codon 27 between the two groups. There was no linkage disequilibrium between codon 16 and 27 of *ADRB2* in both the case ($P = 0{\cdot}088$) and control groups ($P = 0{\cdot}936$). In a logistic regression model, homozygosity of the wild-type allele at codon 16 (Arg/Arg) had an OR of 1·894 for diabetes after adjustment of gender, age and BMI.

In subjects with wild-type of codon 16 in *ADRB2*, this was associated with an earlier onset of type 2 diabetes. We applied survival analysis to estimate the onset age of diabetes between subjects with wild and nonwild genotypes. Wild-type of codon 16 in *ADRB2* was associated with an earlier onset of type 2 diabetes ($P = 0{\cdot}007$, according to log-rank test; Fig. 1).

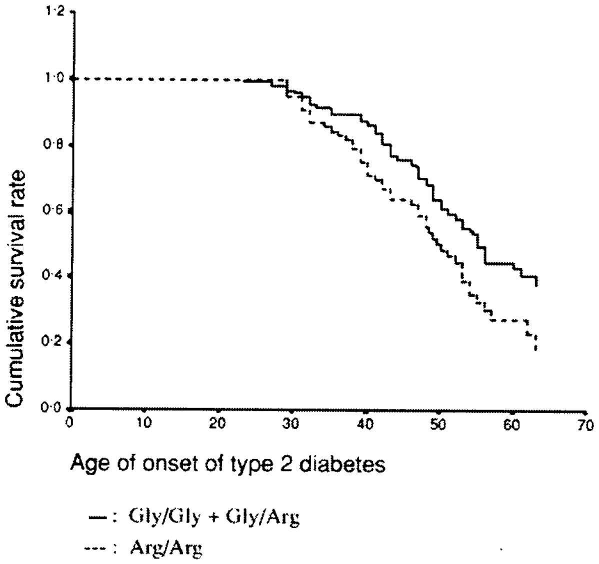

Fig. 1 Subjects with wild type of codon 16 on ADRB2 gene was associated with an earlier onset of type 2 diabetes.

Discussion

In our present study in a Taiwanese population, Arg16Gly substitution of the ADRB2 gene was associated with a lower frequency of type 2 diabetes. More interestingly, the Arg/Arg genotype was associated with an earlier onset of type 2 diabetes. In addition to the known risk factors associated with type 2 diabetes, such as BMI and plasma triglyceride levels, the codon 16 polymorphism of the ADRB2 gene was a strong independent risk factor for type 2 diabetes. In contrast, the codon 27 polymorphism of the ADRB2 gene did not increase the risk factor for type 2 diabetes in this Taiwanese population. Several studies have shown that certain genetic polymorphisms may influence disease prognosis or onset. For example, Arkwright *et al.* reported that patients with cystic fibrosis and a high producer genotype at codon 10 of the TGF-β1 gene had more rapid deterioration in lung function than those with a TGF-β1 low producer genotype. The association of specific glucokinase genotypes with the onset of type 2 diabetes has also been reported. To our knowledge, this study is the first to demonstrate that the Arg16Gly substitution of the *ADRB2* gene is associated with a later onset of type 2 diabetes.

In our study, the Arg16Gly substitution is associated with a lower frequency of type 2 diabetes, which is consistent with previous reports in some ethnic groups. In a Japanese population, Arg16 was strongly associated with obesity in women but not associated with hypertension. On the other hand, homozygous carriers of the Arg16 allele of *ADRB2* gene had an increased risk of hypertension in subjects with type 2 diabetes in a Swedish study. Adipocytes from subjects homozygous for Gly16 had a fivefold higher sensitivity to the β2-selective agonist, terbutaline, than those subjects homozygous for Arg16. Thus, the Gly16 allele could be a protective factor for obesity in female subjects. It has been reported that the basal lipolysis rates decrease by about 50% after weight reduction, but the sensitivity to noradrenaline-stimulated lipolysis *in vitro* increases fivefold after weight reduction. This may be attributed to a decreased activity of hormone-sensitive lipase and an increased sensitivity of β2-adrenoceptors. In addition, plasma concentrations of insulin, noradrenaline and total testosterone decreased and sex hormone binding globulin increased after weight reduction. The increased lipolytic efficiency may be of importance for amelioration of the metabolic complications of obesity. We observed that normal subjects had a higher frequency of the Gly16 allele than diabetic subjects, and the diabetic subjects homozygous for the Arg16 had an earlier onset of disease. The protective effect of the Arg16Gly substitution in the *ADRB2* gene might be explained by the increased lipolytic activity suggested in previous studies. Nevertheless, in any population study, spurious associations may arise from selection bias, population stratification (founder effect), multiple hypothesis testing and subgroup analysis, publication bias, inadequate power and inadequate phenotyping. In our study, 260 subjects were recruited and statistical significance was found, therefore the power was adequate. Moreover, all the subjects in this study were Han Chinese, and genotyping was checked twice. However, family-based studies utilizing a transmission disequilibrium test and formal meta-analyses should be done to provide a definitive answer to this question.

In contrast, our observation on the Gln27Glu polymorphism differs from the published reports. In the Japanese population, the frequency of the variant Glu27 allele of the *ADRB2* was twofold higher in diabetic subjects, and the association between the Glu27 allele and diabetes could be explained by the high frequency of obesity in the subjects with diabetes. In the study on Swedish women, obesity was found to be associated with the codon 27 polymorphism but not with the codon 16 polymorphism. In this study, the frequencies of Gln27Glu substitution between nondiabetic and diabetic subjects were not different, and the Gln27Glu substitution was also not associated with obesity. Moreover, there was no linkage disequilibrium between codon 16 and 27 of *ADRB2* in both case and control groups. The different results from different ethnic groups may be due to genetic heterogeneity.

四、計畫成果自評

在原計畫吾等擬定從脂肪細胞之研究成果而進一步探討在人類疾病之研究，而本研究報告也很成功的反應，在一些與脂肪細胞之分化、代謝相關之基因，極可能與人類肥胖、糖尿病等疾病有關，因此計畫成果部份，除了與原計畫相符，並已將研究成果完成學術期刊之發表〔見參考文獻 1〕。

而系列之研究成果與執行本計畫有相當的關聯，在參考文獻內可以看到此研究方式，應用在多樣化之臨床問題，可供有興趣之研究人員進一步之參考〔見參考文獻 2~4〕。

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The Arg16Gly polymorphism of human β2-adrenoreceptor is associated with type 2 diabetes in Taiwanese people

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Summary

OBJECTIVE The significance of the association of amino terminal polymorphisms in β2-adrenoreceptor (*ADRB2*) with obesity and type 2 diabetes is controversial and differs among ethnic groups. In this study, the association of *ADRB2* with risk and age of onset of type 2 diabetes has been examined in a Taiwanese population.

DESIGN The study design is a case–control study to investigate the impact of the two amino acid polymorphisms in *ADRB2*.

PATIENTS AND MEASUREMENTS This study includes 130 patients with type 2 diabetes [female : male = 1 : 1, age: 52·4 ± 10·0 years; body mass index (BMI): 24·2 ± 2·9 kg/m^2; mean ± SD] and 130 controlled subjects matched for gender, age and BMI with normal glucose tolerance (female : male = 1 : 1, age: 51·7 ± 10·6 years; BMI: 23·9 ± 2·7 kg/m^2). The Arg16Gly and Gln27Glu polymorphisms of *ADRB2* were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) assays. The genotypic and allelic frequencies between two groups were compared and the relationship between the genotypes and clinical phenotypes was examined.

RESULTS A difference in genotypic frequency in the Arg16Gly polymorphism was noted between groups in this gender-, age- and BMI-matched case–control study ($P = 0{\cdot}039$). Multivariate regression analysis revealed that the Arg16Gly polymorphism was the only independent factor for development of type 2 diabetes ($P = 0{\cdot}021$). In addition, we utilized the log-rank test to compare the differences in age of onset between wild-type and nonwild-type polymorphisms. The Arg16Gly polymorphism was independently associated with age of onset in type 2 diabetes ($P = 0{\cdot}017$). There was no difference in the Gln27Glu polymorphism between diabetic and control groups in this study.

CONCLUSIONS In a Taiwanese population, homozygosity of Arg16 in the *ADRB2* gene was associated with a higher frequency (odds ratio 1·87, 95% confidence interval 1·34–2·40) for development of type 2 diabetes. Moreover, this polymorphism was also associated with an earlier onset of type 2 diabetes. However, the Glu27Gln polymorphism had no impact on either BMI or type 2 diabetes in a Taiwanese population.

Obesity is one of the major health issues in developed countries (Mokdad *et al.*, 1999). It is not only associated with increased mortality (Allison *et al.*, 1999; Calle *et al.*, 1999) but is also associated with an increased frequency of type 2 diabetes, gallbladder disease, coronary heart disease, hyperlipidaemia, hypertension and osteoarthritis (Must *et al.*, 1999). It is generally accepted that obesity results from a positive balance in energy homeostasis governed by complex interactions among many genetic and environmental factors (Comuzzie & Allison, 1998; Hill & Peters, 1998). A key process in the energy balance is the mobilization of lipids through lipolysis in adipocytes (Lafontan & Berlan, 1993). Catecholamines play a central role in the regulation of energy expenditure, in part by stimulating lipid metabolism through lipolysis in fat cells (Large *et al.*, 1997). All three known subtypes of β-adrenergic receptors (*ADRB*) promote lipolysis in human adipose tissue *in vivo* (Enocksson *et al.*, 1995; Barbe *et al.*, 1996), although the three *ADRB* subtypes are differently expressed among tissues. *ADRB2* and *ADRB3* are expressed in

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[1] The first two authors contributed equally to this work.

abdominal subcutaneous adipose tissue but *ADRB2* seems to be of greater importance than *ADRB3* for the mobilization of lipids (Lönnqvist *et al.*, 1995). It has also been shown that the sympathetically mediated thermogenic response to stimuli is related to the stimulation of both *ADRB1* and *ADRB2*, but not *ADRB3* (Enocksson *et al.*, 1995). The role of β-adrenergic receptors in the pathogenesis of obesity stems from the studies of the *ADRB3* gene. Associations of the Trp64Arg substitution in the *ADRB3* gene with obesity have been reported in several ethnic groups (Clément *et al.*, 1995; Kadowaki *et al.*, 1995; Kurabayashi *et al.*, 1996), although the role of this variant allele in obesity is still controversial (Gagnon *et al.*, 1996; Yuan *et al.*, 1997).

Although several polymorphisms have been found in the coding region of the *ADRB2* gene in humans, only three of them affect amino acid coding (Reihsaus *et al.*, 1993). These include the rare Thr164Ile variant and two common Arg16Gly and Gln27Glu variants. In Swedish women, Glu27 was found to be a risk factor for obesity (Large *et al.*, 1997). Subjects homozygous for Glu27 had higher indices of obesity, higher body fat, larger fat cell volume and higher fasting insulin concentrations when compared to those with the Gln27 allele (Large *et al.*, 1997). The Glu27 allele was identified as a risk factor for both obesity and diabetes in a Japanese population (Ishiyama-Shigemoto *et al.*, 1999). In contrast, the Gln27 allele is not only a risk factor for diabetes but is also associated with insulin resistance in a family-based population study of another Swedish population (Carlsson *et al.*, 2001). The discrepancy in the reported incidence of the Gln27Glu substitution may be due to differing ethnicity and gender in the various studies. However, no association was found between the Arg16Gly polymorphism and obesity in Swedish women (Large *et al.*, 1997) and in a Japanese population (Ishiyama-Shigemoto *et al.*, 1999). However, the frequency of Gly16 homozygotes was lower in obese Japanese women than in nonobese Japanese women (Ishiyama-Shigemoto *et al.*, 1999). This dichotomy may also be attributable to ethnicity and gender. These studies are summarized in Table 1. Functional studies revealed that the Arg16Gly polymorphism was associated with altered *ADRB2* function, with Gly16 carriers showing a fivefold increase in agonist sensitivity without any change in *ADRB2* expression (Large *et al.*, 1997). A separate study reported that an *ADRB2* with the Gln27Glu substitution expressed in Chinese hamster fibroblasts exhibited altered protein conformation and was completely resistant to agonist-induced receptor down-regulation (Green *et al.*, 1994).

The *ADRB2* gene is a candidate gene for type 2 diabetes and the Gln27Glu and Arg16Gly polymorphisms are the candidate markers for the disease. We conducted the very first study in a Taiwanese population to investigate the role of these two polymorphisms in the pathogenesis of type 2 diabetes.

Materials and methods

Subjects

The study population comprised 130 subjects with type 2 diabetes and 130 nondiabetic controls who were matched for age, sex and body mass index (BMI), and all were unrelated. The nondiabetic subjects (65 men and 65 women), who were recruited from the health examination clinics of National Taiwan University Hospital, had a mean (± SD) BMI of 23·9 ± 2·7 kg/m^2 and a mean age of 51·7 ± 10·6 years. Diabetic subjects (65 men and 65 women; BMI 24·2 ± 2·9 kg/m^2, age 52·4 ± 10·0 years) were recruited from the outpatient clinics of National Taiwan University Hospital. The diagnosis of diabetes mellitus was according to the criteria of the American Diabetes Association (Harris *et al.*, 1997). Most of the diabetic patients received either oral diabetic agents or insulin treatment, and some had diabetic retinopathy, nephropathy and/or neuropathy. All subjects gave written informed consent.

Laboratory and phenotypic characterization of the subjects

The concentrations of plasma glucose, total cholesterol and triglyceride were measured in fasting samples using an autoanalyser (Hitachi 7250 special, Tokyo, Japan).

Table 1 Summary of previous research findings about the association of ADRB2 gene polymorphisms with different phenotypes

Allele	Phenotype	Ethnicity	Gender	Reference
Glu27	Obesity	Swedish	Women	Large *et al.* (1997)
Glu27	Obesity and diabetes	Japanese	Both	Ishiyama-Shigemoto *et al.* (1999)
Gln27	Diabetes and insulin resistance	Swedish	Both	Carlsson *et al.* (2001)
Gly16	No association with obesity	Swedish	Women	Large *et al.* (1997)
Gly16	No association with obesity	Japanese	Both	Ishiyama-Shigemoto *et al.* (1999)
Arg16	Obesity	Japanese	Women	Ishiyama-Shigemoto *et al.* (1999)
Arg16	No association with hypertension	Japanese	Both	Kato *et al.* (2001)
Arg16	Hypertension	Swedish	Both	Bengtsson *et al.* (2001)

Genotypic polymorphisms of the ADRB2 gene

Genomic DNA was extracted from peripheral blood leucocytes using Puregene kits (Promega, Madison, WI, USA). The amplification of *ADRB2* gene sequences was done by polymerase chain reaction (PCR) with the following oligonucleotide primers: 5′-CTT CTT GCT GGC ACG CAA T-3′ and 5′-CCA GTG AAG TGA TGA AGT AGT TGG-3′ for the codon 16 polymorphism, 5′-GGC CCA TGA CCA GAT CAG CA-3′ and 5′-GAA TGA GGC TTC CAG GCG TC-3′ for the codon 27 polymorphism. Annealing temperatures of the primers designed to amplify these regions were 56 °C and 60 °C, respectively. After 35 cycles of amplification, PCR products were analysed in 2% agarose gels to confirm the proper amplification. The size for the codon 16 PCR product amplification with the above primers is 201 bp, and the size for codon 27 product with the above primers is 353 bp. The amplified PCR products were then digested with the addition of BsrD I (codon 16) and Ita I (codon 27). After an incubation at 60 °C (BsrD I) and 37 °C (Ita I) for 1 h, the digested samples were resolved by electrophoresis through a 3% agarose gel and visualized by staining with ethidium bromide. This digestion of codon 27 produced fragments of the following sizes: 27, 55, 97 and 174 bp in Gln27 homozygotes; 27, 55, 97, 174 and 229 bp in Gln27Glu27 heterozygotes; and 27, 97 and 229 bp in Glu27 homozygote (the 27-bp fragment was too small to be resolved on the gel). The digestion of codon 16 produced fragments of the following sizes: 14, 56 and 131 bp in Arg16 homozygotes; 14, 23, 56, 108 and 131 bp in Arg16Gly16 heterozygotes; and 14, 23, 56 and 108 bp in Gly16 homozygotes.

Statistical analyses

Comparisons between groups were made with Student's t-test, χ^2 test, or Fisher's exact test. Logistic regression analysis was used to predict the risk of having type 2 diabetes between subjects with different genotypes. Log-rank test was applied for survival analysis. All statistic methods were performed with the Statistical Package for the Social Sciences (SPSS Inc. Chicago, IL, USA) software, version 10. Linkage disequilibrium between codon 16 and 27 in *ADRB2* was calculated according to CLUMP software (Sham & Curtis, 1995). A P-value of less than 0·05 was considered statistically significant.

Results

Demographic characteristics among type 2 diabetic patients and normal controls

There was no statistically significant difference in the level of plasma triglyceride and cholesterol concentrations between diabetic and nondiabetic groups that were matched for age, sex and BMI (Table 2).

Table 2 Demographic characteristics of diabetic and nondiabetic subjects

	Nondiabetes mellitus	Diabetes mellitus	P-value*
Age (year)	51·7 ± 10·6	52·4 ± 10·0	0·597
Sex (male ; female)	65 : 65	65 : 65	1·000
BMI (kg/m^2)	23·9 ± 2·7	24·2 ± 2·9	0·357
Triglyceride (mmol/l)	1·24 ± 0·75	1·36 ± 0·83	0·252
Cholesterol (mmol/l)	4·63 ± 0·98	4·45 ± 0·80	0·129

Data are given in mean ± SD. *By Student's t-test.

Genotype and allele frequency of the ADRB2 gene polymorphism on codon 16 and codon 27

The observed genotypic frequencies of the Arg16Gly and Gln27Glu polymorphisms were in compliance with the Hardy–Weinberg equilibrium. The arginine and glycine allele frequencies of codon 16 in nondiabetic subjects were 52·7% (137/260) and 47·3% (123/260), respectively. The allelic frequencies of arginine and glycine in diabetic patients were 58·5% (152/260) and 41·5% (108/260), respectively. The glutamine and glutamate allele frequencies of codon 27 in nondiabetic subjects were 92·3% (240/260) and 7·7% (20/260), respectively. The allelic frequencies of glutamine and glutamate in diabetic patients were 91·9% (239/260) and 8·1% (21/260), respectively. The genotypic distribution of codon 16 between the two groups was also statistically significant (Table 3). The wild-type allele was associated with a higher frequency of diabetes in this population, with an odds ratio (OR) of 1·87 [95% confidence interval (CI) 1·34–2·40]. In contrast, no difference was noted in the genotypic distribution and allelic frequencies of codon 27 between the two groups (Table 3). There was no linkage disequilibrium between codon 16 and 27 of *ADRB 2* in both the case (P = 0·088) and control groups (P = 0·936). In a logistic regression model, homozygosity of the wild-type allele at codon 16 (Arg/Arg) had an OR of 1·894 for diabetes after adjustment of gender, age and BMI (Table 4).

In subjects with wild-type of codon 16 in *ADRB2*, this was associated with an earlier onset of type 2 diabetes. We applied survival analysis to estimate the onset age of diabetes between subjects with wild and nonwild genotypes. Wild-type of codon 16 in *ADRB2* was associated with an earlier onset of type 2 diabetes (P = 0·007, according to log-rank test; Fig. 1).

Discussion

In our present study in a Taiwanese population, Arg16Gly substitution of the ADRB2 gene was associated with a lower frequency of type 2 diabetes. More interestingly, the Arg/Arg

Table 3 Genotypic distribution and allelic frequency of the codon 16 and codon 27 polymorphisms in the β2-AR gene between nondiabetic and diabetic subjects

	Nondiabetes mellitus (%)	Diabetes mellitus (%)	*P*-value*	Odds ratio (95% C.I)
Codon 16				
Arg/Arg	31 (23·8)	48 (36·9)	0·039	1·87 (1·34–2·40)
Arg/Gly	75 (57·7)	56 (43·1)		
Gly/Gly	24 (18·5)	26 (20·0)		
Codon 16				
Arg	137 (52·7)	152 (58·5)	0. 1855	1·26(0·91–1·61)
Gly	123 (47·3)	108 (41·5)		
Codon 27				
Gln/Gln	111 (85·4)	110 (84·6)	0·984	0·94 (0·26–1·62)
Gln/Glu	18 (14·8)	19 (14·6)		
Glu/Glu	1 (0·8)	1 (0·8)		
Codon 27				
Gln	240 (92·3)	239 (91·9)	0·872	0·95 (0·46–1·44)
Glu	20 (7·7)	21 (8·1)		

*By Chi-square test.

Table 4 Logistic regression model to predict diabetes mellitus Variables in the Equation

	B	SE	Wald	d.f.	Significance	Exp(B)
Step 1						
ADRB2 (1)	0·639	0·276	5·345	1	0·021	1·894
SEX	0·009	0·251	0·001	1	0·973	1·009
AGE	0·007	0·013	0·304	1	0·582	1·007
BMI	0·036	0·046	0·621	1	0·431	1·037
Constant	−1·431	1·190	1·446	1	0·229	0·239

Dependent variable is diabetic status. *ADRB2*(1): Arg16Gly16 + Gly16Gly16; 0: wild-type of codon 16 Arg/Arg. B, coefficient; SE, standard error; Wald, wald statistic; d.f., degrees of freedom; Exp(B), estimated odds ratio.

genotype was associated with an earlier onset of type 2 diabetes. In addition to the known risk factors associated with type 2 diabetes, such as BMI and plasma triglyceride levels, the codon 16 polymorphism of the ADRB2 gene was a strong independent risk factor for type 2 diabetes. In contrast, the codon 27 polymorphism of the ADRB2 gene did not increase the risk factor for type 2 diabetes in this Taiwanese population. Several studies have shown that certain genetic polymorphisms may influence disease prognosis or onset. For example, Arkwright *et al.* (2002) reported that patients with cystic fibrosis and a high producer genotype at codon 10 of the TGF-β1 gene had more rapid deterioration in lung function than those with a TGF-β1 low producer genotype. The association of specific glucokinase genotypes with the onset of type 2 diabetes has also been reported (Permutt *et al.*, 1992).

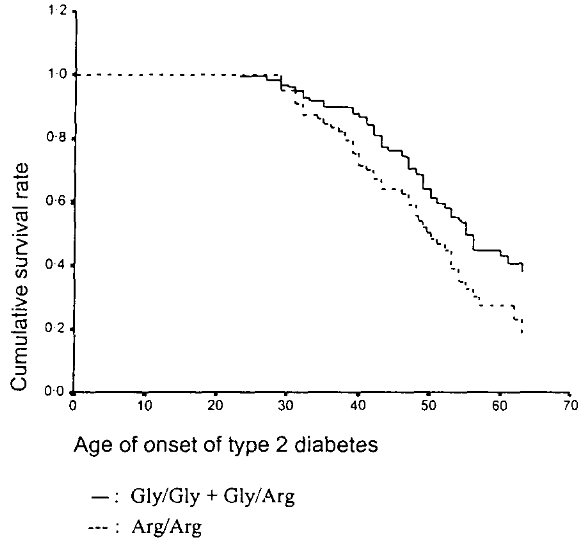

Fig. 1 Subjects with wild type of codon 16 on ADRB2 gene was associated with an earlier onset of type 2 diabetes. Dependent variable is the cumulative diabetes mellitus-free survival rate.

To our knowledge, this study is the first to demonstrate that the Arg16Gly substitution of the *ADRB2* gene is associated with a later onset of type 2 diabetes.

In our study, the Arg16Gly substitution is associated with a lower frequency of type 2 diabetes, which is consistent with previous reports in some ethnic groups. In a Japanese population, Arg16 was strongly associated with obesity in women (Ishiyama-Shigemoto *et al.*, 1999) but not associated with hypertension (Kato *et al.*, 2001; Table 1). On the other hand, homozygous carriers of the Arg16 allele of *ADRB2* gene had an increased risk of hypertension in subjects with type 2 diabetes in a Swedish study (Bengtsson *et al.*, 2001; Table 1). Adipocytes from subjects homozygous for Gly16 had a fivefold higher sensitivity to the β2-selective agonist, terbutaline, than those subjects homozygous for Arg16 (Large *et al.*, 1997). Thus, the Gly16 allele could be a protective factor for obesity in female subjects (Ishiyama-Shigemoto *et al.*, 1999). It has been reported that the basal lipolysis rates decrease by about 50% after weight reduction, but the sensitivity to noradrenaline-stimulated lipolysis *in vitro* increases fivefold after weight reduction. This may be attributed to a decreased activity of hormone-sensitive lipase and an increased sensitivity of β2-adrenoceptors. In addition, plasma concentrations of insulin, noradrenaline and total testosterone decreased and sex hormone binding globulin increased after weight reduction (Reynisdottir *et al.*, 1995). The increased lipolytic efficiency may be of importance for amelioration of the metabolic complications of obesity (Reynisdottir *et al.*, 1995). We observed that normal subjects had a higher frequency of the Gly16 allele than diabetic subjects, and the diabetic subjects homozygous for the Arg16 had an earlier onset of disease. The protective effect of the Arg16Gly substitution in the *ADRB2* gene might be explained by the increased lipolytic activity suggested in previous studies. Nevertheless, in any population study, spurious associations may arise from selection bias, population stratification (founder effect), multiple hypothesis testing and subgroup analysis, publication bias, inadequate power and inadequate phenotyping. In our study, 260 subjects were recruited and statistical significance was found, therefore the power was adequate. Moreover, all the subjects in this study were Han Chinese, and genotyping was checked twice. However, family-based studies utilizing a transmission disequilibrium test and formal meta-analyses should be done to provide a definitive answer to this question.

In contrast, our observation on the Gln27Glu polymorphism differs from the published reports. In the Japanese population, the frequency of the variant Glu27 allele of the *ADRB2* was twofold higher in diabetic subjects, and the association between the Glu27 allele and diabetes could be explained by the high frequency of obesity in the subjects with diabetes (Ishiyama-Shigemoto *et al.*, 1999; Table 1). In the study on Swedish women, obesity was found to be associated with the codon 27 polymorphism but not with the codon 16 polymorphism (Large *et al.*, 1997; Table 1). In this study, the frequencies of Gln27Glu substitution between nondiabetic and diabetic subjects were not different, and the Gln27Glu substitution was also not associated with obesity. Moreover, there was no linkage disequilibrium between codon 16 and 27 of *ADRB2* in both case and control groups. The different results from different ethnic groups may be due to genetic heterogeneity.

In conclusion, the Arg16Gly polymorphism in the *ADRB2* gene was associated with a later age of onset of type 2 diabetes in this Taiwanese population, and this effect was independent of BMI and plasma triglyceride level. However, further studies are required to determine the functional significance of this variant.

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