from Dakar, Senegal, was reported but the study did not include any control group [8]. In fact, this control population can be extrapolated from the initial study by Boutin et al. [6] in which their background population came from the same city (Dakar) and the frequency of the Gly574Ser allele was as high as 33%. Indeed, in our group, 9 of 18 (50%) patients carrying the Gly574Ser allele come from Senegal. This high frequency in Senegal could reflect a founder effect of this mutation in this particular country. Contrary to these previous studies, our diabetic and control populations were all widely distributed around 11 countries of West Africa and three different Caribbean islands. Therefore the 7.2% frequency of the Gly574Ser heterozygous genotype is probably very representative of the sub-Saharan African non diabetic populations.

In conclusion, we gathered a cohort of 80 patients with the pure clinical phenotype of KPAD. The Gly574Ser polymorphism of the HNF-1α gene does not seem associated with this form of diabetes. Further genetic studies are needed to determine the involvement of transcription factors or other candidate genes in the beta-cell dysfunction of KPAD.

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No interactions between polymorphisms in the β_3-adrenergic receptor gene and the PPAR-γ gene on the risk of the insulin resistance syndrome in the Danish MONICA cohort

Keywords Insulin resistance syndrome, type 2 diabetes, genetics, polymorphism, transcription factors.

To the Editor: Multiple mechanisms, including genetic components, are thought to contribute to the pathogenesis of the insulin resistance syndrome (IRS). The genetic factor probably results from the effect of a cluster of variations at multiple genetic loci and genetic interaction, i.e. amplification of allelic effect in the presence of variants at other loci, is likely to be present further illustrating the complexity. The prevalent Ala allele of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-γ (PPAR-γ) gene is associated in many studies with increased insulin sensitivity and a decreased risk of Type 2 diabetes [1]. In line with these observations, we recently showed an association of the Ala/Ala genotype and a decreased propensity to develop the IRS in the Danish Monitoring of trends and determinants in Cardiovascular diseases (MONICA) study population [2]. The Trp64Arg variant in the β3-adrenergic receptor (BAR) gene, another candidate gene for obesity and insulin resistance, has in many studies been linked to features of the IRS. However, many investigations have failed to show such associations. Reasons for these disparities might be insufficient statistical power, ethnic differences and genetic heterogeneity. Interestingly, results from a recent study in Mexican Americans suggest a model in which these two genetic risk factors, having no or modest effect when expressed alone, interact in a synergistic manner on the development of obesity and insulin resistance [3]. We have examined the effect of the Trp64Arg variant expressed alone and in combination

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with genotypes of the Pro12Ala polymorphism on the risk of the IRS in a Danish population. The study group comprised 2656 Danish Caucasian subjects, re-examined from 1993 to 1994 and recruited from a random sample of 4581 Danes [4], who from 1982 to 1983 participated in the MONICA study. The population was selected to represent an equal number of men and women aged 40, 50, 60 and 70 years. Prior to the participation in the study informed consent was obtained for all subjects. The study was approved by the Ethical Committee of Copenhagen and was in accordance with the principles of the Declaration of Helsinki II. After genotyping for both variants and exclusion of Type 2 diabetic patients (fasting plasma glucose >7.0 mmol/l or treated diabetes) 2117 subjects was eligible for analysis. Serum insulin was measured by enzyme-linked two-site immunoassay specific for intact insulin and without cross-reactivity of proinsulin. Insulin resistance was calculated using homeostasis model assessment (HOMA) analysis [5]. Genotyping was done as previously described [2, 6]. Chi-square analysis and Fisher's exact test when appropriate were applied to test for statistical significance of differences in allele frequencies. Differences in continuous variables were assessed by analysis of covariance, adjusted for age, sex and BMI using a general linear model. Statistical significance was tested using interaction as well as recessive models. Residuals of the variables (or the transformed variables) were confirmed to be normally distributed. A p value of less than 0.05 was considered statistically significant. SPSS for Windows v. 9.0. was used. Using the criteria defined by the European Group for the Study of Insulin Resistance (EGIR) [7] the study population was stratified in an IRS group (n=269) and a group not having this syndrome (non-IRS) (n=1848). The allelic frequency of the Trp64Arg polymorphism was 7.1% (95% CI: 4.9–9.3%) in the IRS group and 7.2% (6.4–8.0%) in the non-IRS group (p=0.5). Within the total study population there were no differences across the three groups of genotypes with respect to body composition (BMI or waist), serum lipids, plasma glucose, serum insulin or HOMA estimates of insulin secretion or insulin sensitivity, neither carrying out interaction analysis nor the recessive model (Table 1). We next evaluated the effect of both the Trp64Arg and the Pro12Ala variant, of which nine combinations were possible. The impact of the Ala/Ala carriers in this study group was recently revealed [2]. This genotype is distributed mainly on the *Trp/Trp* genotype of BAR as only one subject carried the *Ala/Ala* + *Arg/Arg* genotype and two subjects the *Ala/Ala* + *Trp/Arg* genotype. Thus, in order to obtain sufficient statistical power the nine genotype groups were collapsed into four (Table 1), which also allowed for direct comparison with the previous report in Mexican Americans. There was no difference in the combined genotype distribution between the IRS group [(number (frequency)] wild-type [167 (65.5%)], only Trp64Arg variant [25 (9.8%)], only Pro12Ala variant [53 (20.8%)], both variants [10 (3.9%)] and the non-IRS group [1141(64.2%), 179 (10.1%), 399 (22.5%) and 58 (3.3%), respectively] (p=0.89). Furthermore, as it seems we were unable to show any statistically significant difference across the four groups of genotypes with respect to any of the examined variables (Table 1).

Carriers of the *Arg/Arg* genotype of the Trp64Arg variant have in another Danish study of 380 young (25 years of age) healthy subjects previously shown to be insulin resistant as estimated from an IVGTT [6]. However, the conclusion was based on three carriers of this genotype. In the present study the power to detect effect at the same level of the *Arg/Arg* genotype on the HOMA estimate of insulin sensitivity was calculated to about 85%, indicating low risks of false negative results. Clinical studies of the Pro12 Ala variant have been more consistent relating the variant to increased insulin sensitivity.

Table 1. Clinical and biochemical data of 2117 non-diabetic Danish subjects classified in accordance to their genotype of the Trp64Arg variant of the β_3-Adrenergic receptor (B3AR) gene and to the combined genotype of the Trp64Arg variant and the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor (PPAR)-γ gene

B3AR genotype	B3AR genotype			p	Combined genotypes				p
	Trp/Trp	*Trp/Arg*	*Arg/Arg*		No variants	Trp64Arg variant only	PPAR variant only	Both variants	
n (men/women)	905/926	146/122	7/11	0.19	645/663	114/90	220/232	31/37	0.22
n (40 /50/60/70 years)	529/519/456/327	81/66/71/50	7/5/4/2	0.84	372/377/325/234	62/50/57/35	141/126/109/76	21/19/15/13	0.93
BMI (kg/m^2)	25.8 (4.1)	25.9 (3.8)	26.5 (4.4)	0.59 (0.30)	25.8 (4.2)	26.0 (3.9)	25.7 (3.8)	25.3 (3.8)	0.91 (0.15)
Waist (cm)	87.1 (12.3)	87.9 (12.1)	87.8 (12.5)	0.38 (0.77)	87.1 (12.4)	88.2 (11.6)	87.9 (11.9)	86.7 (12.9)	0.99 (0.48)
Systolic BP (mmHg)	1288 (19.2)	127.9 (18.1)	129.8 (23.3)	0.40 (0.49)	128.7 (19.4)	127.7 (18.9)	128.3 (18.3)	128.3 (17.4)	0.85 (0.27)
Diastolic BP (mmHg)	82.0 (10.5)	81.4 (10.2)	83.3 (14.4)	0.53 (0.54)	82.0 (10.7)	81.4 (10.4)	81.6 (9.7)	81.6 (11.0)	0.55 (0.89)
S-total cholesterol (mmol/l)	6.1 (1.1)	6.1 (1.1)	6.3 (1.0)	0.69 (0.44)	6.1 (1.1)	6.1 (1.0)	6.2 (1.1)	6.3 (1.1)	0.68 (0.69)
S-LDL cholesterol (mmol/l)	4.0 (1.0)	4.0 (1.0)	4.1 (1.0)	0.86 (0.61)	4.0 (1.0)	4.0 (0.9)	4.1 (1.1)	4.1 (1.0)	0.55 (0.89)
S-HDL cholesterol (mmol/l)	1.5 (0.4)	1.4 (0.4)	1.5 (0.4)	0.99 (0.94)	1.5 (0.4)	1.4 (0.4)	1.5 (0.4)	1.5 (0.5)	0.15 (0.69)
S-triglyceride (mmol/l)	1.4 (1.0)	1.4 (1.0)	1.6 (0.7)	0.35 (0.24)	1.4 (0.9)	1.4 (1.1)	1.4 (1.0)	1.4 (0.8)	0.78 (0.40)
P-glucose (mmol/l)	4.8 (0.5)	4.8 (0.5)	4.7 (0.4)	0.37 (0.59)	4.7 (0.5)	4.8 (0.5)	4.8 (0.5)	4.8 (0.5)	0.28 (0.82)
S-insulin (pmol/l)	35 (28)	36 (27)	42 (33)	0.91 (0.70)	36 (27)	36 (25)	35 (30)	38 (34)	0.36 (0.30)
HOMA insulin resistance	1.29 (1.13)	1.32 (1.15)	1.51 (1.21)	0.89 (0.68)	1.29 (1.10)	1.31 (1.05)	1.29 (1.23)	1.43 (1.47)	0.09 (0.39)
HOMA insulin secretion	99.7 (80.5)	94.2 (58.0)	117.7 (88.7)	0.81 (0.58)	100.3 (79.4)	100.0 (58.5)	98.7 (88.2)	91.0 (59.3)	0.41 (0.62)

Data represent means (±SD). All plasma and serum values were obtained in the fasting state. p values were obtained using a general linear model. p values out of parentheses refer to interaction analysis comparing all genotypes (B3AR or combination) and p values in parentheses refer to analyses using recessive models comparing *Arg/Arg* carriers with *Trp/Trp* and *Trp/Arg* in the B3AR study and "both variants" with the three other groups of genotypes in the combination study

Somewhat paradoxically the variant has also been linked to obesity and recently another Danish study of young men showed divergent modulating effects of the variant on BMI depending on the degree of obesity [8]. Hence, on the basis of variable interaction with other genetic or environmental factors, the polymorphism might express variable effects in different study subgroups. In Mexican Americans the variant was recently shown to be associated with obesity but only in the presence of the Trp64Arg variant of BAR, pointing to the interesting conclusion that the effect observed of the Pro12Ala variant is due to a genetic interaction [3]. We were, however, unable to confirm this hypothesis since carriers of both gene variants did not differ from wild-type carriers or carriers of either polymorphisms with respect to any of the examined phenotypes. Importantly our conclusion is based on statistical power calculations of more than 80%, thereby minimizing the risk of statistical type II errors.

In conclusion, in a Danish population based study group of more than 2100 subjects the Trp64Arg polymorphism of the BAR gene does not associate with insulin resistant phenotypes nor do our results suggest that the variant interacts with the Pro12Ala variant of the PPAR-γ gene on these abnormalities.

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Abbreviations: IRS, insulin resistance syndrome; PPAR-γ, peroxisome proliferator-activated receptor-γ; (BAR), β3-adrenergic receptor; HOMA, homeostasis model assessment; MONICA, Monitoring of trends and determinants in cardiovascular diseases