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The Val985Met Insulin-Receptor Variant in the Danish Caucasian Population: Lack of Associations with Non–Insulin-Dependent Diabetes Mellitus or Insulin Resistance

To the Editor:

A recent study in a Dutch population sample has shown a strong association between a Val985Met missense mutation in the insulin-receptor gene and the common form of non–insulin-dependent diabetes mellitus (NIDDM) (5.6%, vs. 1.3% in the control population). The authors also conclude that this Val985Met variant in the insulin receptor associates with hyperglycemia ('t Hart et al. 1996).

The Val985Met missense mutation in exon 17 of the insulin-receptor gene first was found in sporadic form in cohorts of NIDDM patients and later in control subjects (O'Rahilly et al. 1991, 1992), although without consequences for the insulin-receptor phosphorylation, and, investigated in vivo by overexpression in Chinese hamster-ovary cells, this Val985Met insulin-receptor variant had normal phosphorylation in response to insulin (Flier et al. 1993). When studied in NIDDM pedigrees, this Val985Met insulin receptor variant did not segregate with NIDDM, although carriers of the polymorphism tended to have higher levels of postglucose load levels of plasma glucose (Elbein et al. 1993).

In an attempt to replicate the Dutch findings in the Danish Caucasian population, we have determined the distribution of the three known polymorphisms in exon 17 of the insulin-receptor gene in 254 unrelated Danish Caucasian NIDDM patients recruited from the outpatient clinic at Steno Diabetes Center (Copenhagen) and in 243 age-matched, unrelated, and glucose-tolerant Danish Caucasian control subjects traced in the Danish Central Population Register and living in the same area of Copenhagen as the NIDDM patients. Furthermore, we have estimated the potential physiological impact of the Val985Met receptor variant on the whole-body insulin-sensitivity index and on both glucose-stimulated serum insulin and C-peptide release in 380 study participants randomly recruited from a population of young individuals 18–32 years of age, who, in 1979–80 and again in 1984–85, as children, had participated in blood-pressure surveys in a representative and specified part of Copenhagen city and who are characterized by an intravenous glucose-tolerance test in combination with tolbutamide injection and who were analyzed in accordance with the Bergman minimal model (Clausen et al. 1996). In this study material we previously have reported changes in the whole-body insulin-sensitivity index and glucose-stimulated insulin secretion in individuals carrying genetic variants in key proteins in the insulin-signaling cascade (Clausen et al. 1995; Hansen et al. 1995).

Genotyping was performed by PCR amplification of the entire exon 17 of the human insulin-receptor gene, from 0.1 μg of genomic DNA, by use of 5 pmol each of intronic sense primer, 5′-tgggtggaaggtggcgtcaga-3′, and antisense primer, 5′-tcaggaaagccagcccatgtc-3′, yielding a 353-bp DNA segment (Seino et al. 1990). *Bsa*AI and *Rsa*I restriction analyses of 10 μl of PCR product were performed in separate 20-μl reactions. All the reactions were analyzed on a 2% agarose gel. Exon 17 of the insulin-receptor gene contains two sites for *Bsa*AI (ACGT) and only a single site for *Rsa*I (GTAC). One of the *Bsa*AI sites is disrupted by the Val985Met (GTG/ATG), as well as by the TAC984TAT polymorphism, and the other is disrupted by the CAC1058CAT polymorphism, whereas the *Rsa*I site is disrupted only by the TAC984TAT polymorphism. Therefore, depending on the genotype, *Bsa*AI restriction digestion of exon 17 (353 bp) gives a combination of four major visible

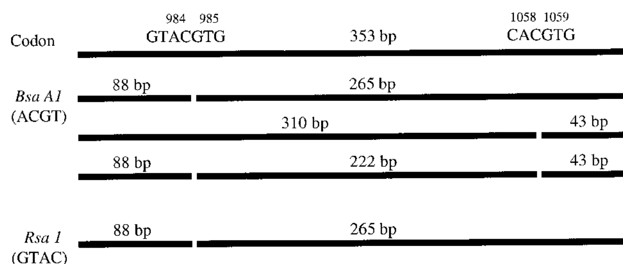

Figure 1 Diagram showing both the size of the PCR-amplified DNA segment of exon 17 in the insulin-receptor gene and the resulting DNA fragments after digestion with the restriction endonucleases *Bsa*AI and *Rsa*I.

bands—353 bp (uncut), 310 bp, 265 bp, and 222 bp—but all bands exist together only in incompletely digested samples. However, since *Bsa*AI cannot distinguish the TAC984TAT from the Val985Met (GTG/ATG) polymorphism, a *Rsa*I restriction digestion also is needed, giving two major bands—353 bp and 265 bp (fig. 1). Carriers of the TAC984TAT polymorphism or the Val985Met (GTG/ATG) polymorphism display either a 353-bp or 310-bp band in the *Bsa*AI digestion, but, if they are wild type in the *Rsa*I digestion (only one band, at 265 bp), they are considered carriers (heterozygous) of the Val985Met (GTG/ATG) polymorphism. All the samples that had a 310-bp or a 353-bp fragment in the *Bsa*AI restriction and a 353-bp fragment in the *Rsa*I restriction were sequenced as described elsewhere (Hansen et al. 1995), in order not to conceal any coupling between the Val985Met (GTG/ATG) polymorphism and the TAC984TAT polymorphism. Samples with a 353-bp or 265-bp band, either together with (heterozygous) or without (homozygous) a 222-bp band, were considered to be carriers of the CAC1058TAT polymorphism. In the 254 NIDDM patients, the carrier frequency of the Val985Met insulin receptor was .020 (.95 confidence interval [95% CI] .003–.037) and .016 (95% CI 0–0.037) in the 243 control subjects (table 1).

In the diabetic population, the carrier frequency of the TAC984TAT polymorphism was .177 (95% CI .130–.224), whereas it was .103 (95% CI .065–.141; $P < .01$) in the control population (obtained by χ^2 analysis). This finding, however, we consider to be insignificant, for two reasons. First, because of the comparison of multiple polymorphisms, the normally accepted significance level of 5% will be 1.7% when adjusted for comparison of the three different polymorphisms. Thus, the association of TAC984TAT with NIDDM reaches only borderline significance. Second, there was no difference in the reported prevalence of this polymorphism in the Dutch NIDDM and control populations ('t Hart et al. 1996). We found no compound-heterozygous carriers of both the Val985Met and the TAC984TAT polymorphisms.

The third polymorphism (CAC1058CAT) had a carrier frequency of .299 (95% CI .243–.355) in the NIDDM population and .314 (95% CI .256–.372) in the control subjects. All three polymorphisms in exon 17 of the insulin-receptor gene were in Hardy-Weinberg equilibrium.

We could not see any impact of the Val985Met receptor on the fasting, 1-h, and 2-h post–glucose load plasma glucose or serum insulin levels, neither in the population of NIDDM subjects nor in the control subjects (data not shown). Serum insulin levels were determined by enzyme-linked immunosorbent assay with a narrow specificity excluding des(31,32)-proinsulin and intact proinsulin, when the DAKO insulin kit was applied, with overnight incubation, as described elsewhere (Andersen et al. 1993).

In the population-based sample of 380 young healthy Danes, the carrier frequency of the Val985Met polymorphism was .021 (95% CI .006–.036), and the carrier frequency of the TAC984TAT polymorphism was .128 (95% CI .094–.162); the carrier frequency of the CAC1-058CAT polymorphism was .332 (95% CI .284–.380) in this cohort (table 2). None of these frequencies differed statistically from either that in the NIDDM cohort or that in the control cohort. The eight young healthy

Table 1

Distribution of Insulin-Receptor Genotypes in the Danish Caucasian Population

	No. of Individuals					
	NIDDM Patients			Control Subjects		
Codon	Wild Type	Heterozygous	Homozygous	Wild Type	Heterozygous	Homozygous
Val985Met (gtg/atg)	249	5	0	239	4	0
TAC984TAT*	209	43	2	218	25	0
CAC1058CAT	178	69	7	166	70	7

* $P < .01$, for carrier frequency.

Table 2

Distribution of Insulin-Receptor Genotypes in 380 Young Healthy Caucasians

	No. of Individuals with Genotype			
Codon	Wild Type	Heterozygous	Homozygous	Total No. of Carriers
Val985Met (gtg/atg)	372	8	0	8
TAC984TAT	334	44	2	46
CAC1058CAT	256	113	11	124

carriers of the Val985Met receptor variant had a whole-body insulin-sensitivity index (Si) comparable to that of the noncarriers, and no difference was seen in fasting values of plasma glucose, serum insulin, and serum C-peptide or in first-phase insulin and C-peptide secretion after intravenous glucose loads (table 3).

So, in the Danish Caucasian population, young healthy carriers of the Val985Met receptor variant had a normal whole-body insulin-sensitivity index and showed no signs of compensatory changes in insulin secretion. Moreover, in contrast to the Dutch findings, carriers also had plasma glucose levels completely indistinguishable from those of noncarriers, in all three groups studied. We suggest that 't Hart et al. (1996) may have overinterpreted the potential influence of the Val985Met polymorphism on the plasma glucose values—and that this occurred for two reasons: first, the authors used "random" plasma values—that is, not fasting plasma glucose values—and the glucose values are not adjusted for serum insulin levels (both insulin deficiency and eating affect plasma glucose levels); second, 't Hart et al. (1996) analyze diabetic and nondiabetic carriers of the Val985Met variant together, rather than analyzing diabetic noncarriers versus diabetic carriers and nondiabetic carriers versus nondiabetic noncarriers. Since the diabetics represent 9/16 of all the carriers but only 153/531 of the noncarriers, the hyperglycemic diabetic carriers will dominate in the analysis and hence tend to give a false impression of an association between the Val985Met variant and hyperglycemia.

Table 3

Mean and SD for Physiological Variables, according to Codon 985 Insulin-Receptor Genotype, in 380 Young Healthy Caucasians

	Mean (SD) for Genotype		
Variable	Val985Val	Val985Met	*P*
No. of males/no. of females	182/190	4/4	
Fasting plasma glucose (mmol/liter)	4.98 (.48)	5.04 (.38)	.74
Fasting serum insulin (pmol/liter)	37 (22)	35 (15)	.86
Fasting serum C-peptide (pmol/liter)	474 (161)	441 (89)	.80
First-phase insulin (area under curve 0–8 min) (pmol/min/liter)[a]	2,247 (1,598)	2,131 (1,104)	.95
First-phase C-peptide (area under curve 0–8 min) (pmol/min/liter)[a]	7,076 (3,341)	7,404 (2,670)	.60
Insulin-sensitivity index (10^{-5} liter/pmol/min)	12.6 (2.3)	15.4 (9.4)	.71

[a] Estimated as the incremental area under the curve during the first 8 min after intravenous injection of 0.3 g of 50% glucose/kg body weight. For statistical comparison, the Mann-Whitney test was applied.

Both the prevalence (~2%) of the Val985Met polymorphism in the different groups studied in the Danish Caucasian population and the lack of association with NIDDM are in accordance with earlier reports (O'Rahilly et al. 1992; Elbein et al. 1993). Also, the fact that the nondiabetic carriers of the Val985Met polymorphism have intermediary metabolism that is indistinguishable from that of the noncarriers is consistent with previous studies of overexpression of this mutant receptor, studies that showed normal autophosphorylation, normal receptor internalization, and even an increased downstream phosphorylation of insulin-receptor substrate-1 (Flier et al. 1993; Strack et al. 1996). In conclusion, in the Danish Caucasian population, the Val985Met polymorphism of the insulin receptor is not associated with an impaired insulin-sensitivity index or with NIDDM.

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Absence of Mutations Raises Doubts about the Role of the 70-kD Peroxisomal Membrane Protein in Zellweger Syndrome

To the Editor:

The 70-kD peroxisomal membrane protein (PMP70) was first purified from rat liver tissue, and analysis of its sequence showed that it belonged to a family of ATP-binding proteins, the ABC transporters (Kamijo et al. 1990). In rat liver, PMP70 is induced by peroxisomal proliferators, and it was proposed that the protein may be part of the import machinery for peroxisomal proteins (Kamijo et al. 1990). Import of proteins into the peroxisome is defective in patients with a range of clinical phenotypes, including those with Zellweger syndrome, neonatal adrenoleukodystrophy, and infantile Refsum disease. Complementation analysis has indicated that this group of disorders can be caused by defects in a number of different genes (Shimozawa et al. 1993; Moser et al. 1995) and that all of the aforementioned phenotypes can occur within the one complementation group. In 1992, Gärtner et al. (1992*a*) proposed that defects in the gene for PMP70 are the cause of the peroxisomal assembly defect in patients belonging to complementation group 1 (Kennedy-Krieger Institute nomenclature). This hypothesis was based on the identification of both a donor splice-site mutation in a single patient and a missense mutation in two affected sibs, all belonging to this complementation group. However, mutations were not identified on the other PMP70 allele from these individuals, nor on alleles from a further 19 complementation group 1 patients (Gärtner et al. 1992*a*). Further support for the involvement of PMP70 in peroxisomal biogenesis, but not necessarily in complementation group 1, has come from transfection studies using a peroxisome-deficient Chinese hamster ovary (CHO) cell mutant carrying a point mutation in another peroxisomal membrane protein, PMP35 (Gärtner et al. 1994). Overexpression of normal PMP70 cDNA was able to overcome the peroxisomal biogenesis defect caused by the defect in PMP35. However, overexpression of a PMP70 mutant cDNA, carrying the predicted 23-bp insertion resulting from the donor splice-site mutation found in one of the patients, did not complement the PMP35 defect (Gärtner et al. 1994).