Am. J. Hum. Genet. 59:1119–1125, 1996

# Association of the Insulin-Receptor Variant Met-985 with Hyperglycemia and Non–Insulin-Dependent Diabetes Mellitus in the Netherlands: A Population-Based Study

L. M. 't Hart,[1] R. P. Stolk,[2] R. J. Heine,[3] D. E. Grobbee,[2] F. E. E. van der Does,[3] and J. A. Maassen[1]

[1]Department of Medical Biochemistry, Sylvius Laboratories, Leiden University, Leiden; [2]Department of Epidemiology and Biostatistics, Erasmus University Medical School, Rotterdam; and [3]Institute for Research in Extramural Medicine, Free University, Amsterdam

## Summary

**One of the characteristics of non–insulin-dependent diabetes mellitus (NIDDM) is the presence of insulin resistance. Most NIDDM patients have a normal sequence of the insulin receptor, indicating that, if insulin-receptor mutations contribute to the development of NIDDM, they will be present only in a minor fraction of the NIDDM population. The goal of the present study was to examine whether insulin-receptor mutations contribute to the development of NIDDM. We examined 161 individuals with NIDDM and 538 healthy controls from the population-based Rotterdam study for the presence of mutations in the insulin-receptor gene by SSCP. A heterozygous mutation changing valine-985 into methionine was detected in 5.6% of diabetic subjects and in 1.3% of individuals with normal oral glucose tolerance test. Adjusted for age, gender, and body-mass index, this revealed a relative risk for diabetes of 4.49 (95% confidence interval 1.59–12.25) for Met-985 carriers. When the total study group was analyzed, the prevalence of the mutation increased with increasing serum glucose levels (test for trend $P < .005$). We conclude that the Met-985 insulin-receptor variant associates with hyperglycemia and represents a risk factor for NIDDM.**

## Introduction

Resistance to the biological effects of endogenous insulin is a prominent feature of non–insulin-dependent diabetes mellitus (NIDDM). In addition, a diminished insulin secretion by pancreatic beta cells is present. Both inherited and lifestyle factors contribute to the pathogenesis of NIDDM. A number of studies have shown that peripheral insulin resistance is an early marker for the development of NIDDM (Beck-Nielsen and Groop 1988; Eriksson et al. 1989; Warram et al. 1990; Vaag et al. 1992; Yki-Järvinen 1994; Feskens et al. 1994). These observations suggest that genetic lesions that associate with insulin resistance are risk factors for NIDDM. In the majority of NIDDM cases, the mode of inheritance is not Mendelian and polygenic factors are involved. In some diabetic subtypes, however, diabetes associates with single-gene defects and an involvement of mutations in the glucokinase gene (Hattersly et al. 1992; Vionnet et al. 1992) and in mtDNA (van den Ouweland et al. 1992; Maassen and Kadowaki 1996) have been found. In addition, glucose intolerance associates with a number of autosomal recessive diseases with Mendelian inheritance, such as Prader-Willi syndrome, Werner syndrome, and ataxia telangiectasia (Garvey and Birnbaum 1993). These findings indicate the ability of different single-gene lesions to contribute to the pathogenesis of diabetes.

Mutations in the insulin-receptor gene are candidates to contribute to the development of insulin resistance and NIDDM. Missense mutations in the insulin receptor are found in phenotypically different syndromes of insulin resistance. Most mutations in the extracellular part of the receptor are reflected in hyperinsulinemia without a marked tendency to glucose intolerance (Taylor et al. 1992). On the other hand, some missense mutations in the cytoplasmic part of the receptor associate with insulin resistance and diabetes. The majority of those mutations are found in exons 17–20, and some exhibit a dominant character (Taylor et al. 1992; Imamura et al. 1994).

Sequence analysis of the insulin receptor in a limited number of diabetes patients has shown a wild-type insulin-receptor sequence indicating that, in case receptor mutations contribute to NIDDM, they will be present only in a fraction of NIDDM patients (Moller et al. 1989; Taylor et al. 1992).

We have examined by SSCP the exons 17–20 of the insulin-receptor gene for mutations that associate with

Received May 29, 1996; accepted for publication August 12, 1996.

Address for correspondences and reprints: Dr. J. Antonie Maassen, Department of Medical Biochemistry, Sylvius Laboratory, Wassenaarseweg 72, 2333 AL Leiden, the Netherlands.

© 1996 by The American Society of Human Genetics. All rights reserved.

0002-9297/96/5905-0022$02.00

diabetes. Diabetic and nondiabetic individuals were selected from the population-based Rotterdam study. All participants were ⩾55 years of age. Glucose homeostasis in the subjects was assessed by oral glucose-tolerance tests (OGTTs).

## Subjects and Methods

### Subjects

The Rotterdam study is a population-based cohort study of determinants for chronic disabling diseases in the elderly. An outline of this study has been published previously (Hofman et al. 1991). The participants came to the research center throughout the day. Anthropometric measurements were performed, and blood was drawn by venepuncture. Subjects without blood glucose–lowering medication received orally 75 g of glucose. Two hours later, a second blood sample was obtained, and glucose levels were measured in both samples. Diabetes mellitus was defined as the use of blood glucose–lowering medication or at least one glucose value of 11.1 mmol/liter. All subjects had provided informed consent, and the study was approved by the medical ethics committee of the Erasmus University Medical School. Overall, 7,983 participants were examined in the Rotterdam study (response rate 78%). For the present study, DNA from two randomly sampled groups, one with diabetes mellitus ($n$ = 161) and one without diabetes mellitus ($n$ = 538) was examined.

### Methods

Chemicals were of analytical grade. Restriction enzymes were from New England Biolabs, Boehringer, or Pharmacia. *Taq* polymerase was from Perkin Elmer. Amplifications of exons 17–20 was by use of the primer set according to the method of Seino et al. (1990) and according to conditions described by van der Vorm et al. (1992). DNA sequencing of amplified DNA and SSCP were performed as described elsewhere (van der Vorm et al. 1992; Krook et al. 1994). Allele-specific oligonucleotide hybridization, to discriminate the Val-985 allele from the Met-985 allele, was performed using the oligonucleotide 5′-TCT GTG TAA GTG CCG GAC GA for the Val-allele (GTG) and 5′-TCT GTG TAA ATG CCG GAC GA for the Met-allele (ATG) as described earlier by van der Vorm et al. (1992). To avoid any interference by the silent Tyr-984 polymorphism (TAT/TAC), an additional mismatch at the third position of this codon was introduced (by the TAA codon) in the oligos for the Met-985 allele and the Val-985 allele.

### Data Analysis

Clinical characteristics were compared between subjects with and without the Met-985 allele by $t$-test for continuous variables and by Mann-Whitney rank-sum test for categorical variables. Multiple linear regression analysis was used to control for possible confounders, notably age, gender, and body-mass index. The characteristics were also compared within the group of subjects with known diabetes. In addition, the association of the Met-985 allele and the presence of diabetes mellitus was estimated by logistic regression analysis with odds ratios (with the 95% confidence interval) as an approximation of relative risk.

## Results

### Identification of the Met-985 Variant by SSCP

Exon 17 was amplified using leukocyte DNA, and examination for sequence variants by SSCP revealed specific patterns in a subgroup of individuals. DNA sequencing showed that one variant was due to the presence of an ATG for GTG, yielding a Met-for-Val substitution at position 985 in the insulin-receptor beta chain. Individuals were heterozygous for this mutation. The other variant resulted from a silent variation in the His-1058 codon. Figure 1 shows an example of the various SSCP patterns, together with the DNA sequence analysis of the Val/Met-985 alleles. The SSCP pattern is not affected by a silent polymorphism in the flanking Tyr-984 codon (TAT versus TAC). The presence of the TAC codon creates a site for *Rsa*I (GTAC). DNA was additionally examined by *Rsa*I cleavage. Allele frequencies for the TAT allele were ~7% in both Met-985 and Val-985 individuals.

### Detection of the Met-985 Variant by Oligonucleotide Hybridization

SSCP analysis was performed on ~100 diabetic and 100 control individuals. To analyze the presence of the Met-985 variant in larger numbers of individuals, we used allele-specific oligonucleotide hybridization to distinguish between the Val and Met alleles. Because of the silent variation in the Tyr-984 codon, we introduced in the Val/Met allele–specific oligonucleotides an additional mutation that mismatches with both Tyr-984 codons, at the site of the silent polymorphism. When tested on amplified DNA samples with known DNA sequences, it was found that these oligonucleotides recognized specifically either the Val-985 or the Met-985 variant under the appropriate conditions, irrespective of the variants at codon 984. We examined 161 subjects with NIDDM and 538 control subjects from the Rotterdam study by allele-specific oligonucleotide hybridization. Nine (5.6%) individuals from the diabetic group were found heterozygous for the Met-985 allele, whereas in the control group seven (1.3%) individuals were positive. Figure 2 gives an example of the specificity of the allele-specific hybridization. This method and SSCP

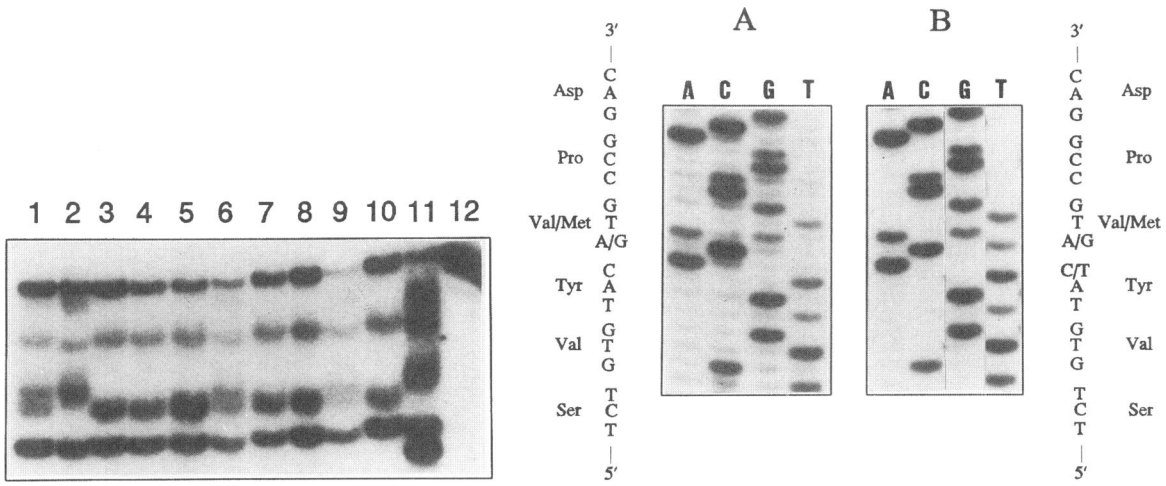


**Figure 1** *Left,* SSCP analysis of amplified exon 17 from 11 individuals. Subsequent DNA analysis showed that variants 1, 6, and 9 are heterozygous for the His-1058 CAT codon, variant 2 being homozygous for the CAT codon. Individuals 3–5, 7–8, and 10 are homozygous for the His-1058 CAC codon. All these individuals are homozygous for Val-985. Individual 11 is heterozygous for Met-985 and homozygous for the His-1058 CAC codon. Lane 12 represents an undenatured SSCP sample. *Right, A,* heterozygosity for Met/Val-985, and homozygosity for the Tyr-984 TAC codon. *B,* Heterozygosity for Met/Val-985 and heterozygosity for Tyr-984 TAC/TAT.

yielded identical results on the distribution of the Met-985 allele.

### *Frequency of Silent Receptor Polymorphisms in Control and Diabetic Groups and Association with Clinical Parameters*

To examine the distribution of known receptor variants over the control and diabetic groups, two silent insulin-receptor mutations were considered. The allele frequency of a silent variation of the His-1058 codon in exon 17 (CAC/CAT) was analyzed by oligonucleotide hybridization. The allele frequencies among diabetic and control individuals was not significantly different (CAT-allele frequency in subjects with diabetes, 14.9%; in controls, 13.8%; $P > .5$). The other polymorphism is a silent *Rsa*I polymorphism in exon 3, which was assayed by *Rsa*I cleavage of a PCR fragment containing exon 3. The allele frequency of this polymorphism was 22.2% and 22.9% in subjects with and without diabetes mellitus, respectively ($P > .5$). None of these two polymorphisms was associated with age, gender, body-mass index, or glucose level ($P > .20$).

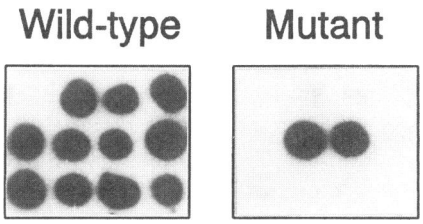


**Figure 2** Allele-specific oligonucleotide hybridization. In the wild-type panel, the filter was hybridized with a radiolabeled oligonucleotide for Val-985. In the mutant panel, after removal of the probe, the filter was reprobed with the oligonucleotide for Met-985. Approximately 0.1 μg of amplified exon 17 DNA, from 11 individuals, was spotted on a nitrocellulose filter, followed by consecutive hybridization of the filter with $^{32}$P-labeled oligonucleotides specific for Val-985 and Met-985 sequence.

### *Associations of the Met-985 Allele with Clinical Characteristics*

Table 1 shows the clinical characteristics of the study population. Both serum glucose level and the prevalence of diabetes mellitus are significantly higher in the Met-985 group. These differences remained after adjusting for age, gender, and body-mass index. Figure 3 shows that the prevalence of the Met-985 allele increases linearly with increasing glucose levels and that a trend analysis shows statistical significance ($P < .005$).

In the group of subjects with diabetes mellitus 5.6% (9/161) of the individuals was heterozygous for the mutation, whereas in the nondiabetic group this value was 1.3% (7/538). The relative risk of Met-985 for the presence of diabetes was 4.49 (95% confidence interval 1.65–12.26), which did not change essentially after adjustment for age, gender, and body-mass index (relative risk 4.42; 1.59–12.25).

Within the group of subjects with diabetes mellitus, those with the Met-985 allele were slightly younger and used antidiabetes medication more often. Because of the small numbers, these differences did not reach statistical significance, but they suggest an earlier age at onset. In addition, among diabetics, those with the mutation had a trend to higher random glucose (13.9 vs. 11.9 mmol/

**Table 1**

**Characteristics of the Study Group**

| Characteristic | Whole Group | Insulin-Receptor Variants | | *P*-Value |
|---|---|---|---|---|
| | | Val-985 | Met-985 | |
| Number | 699 | 683 | 16 | ... |
| Age (years) | 66.6 ± 7.0 | 66.6 ± .3 | 65.9 ± 1.4 | .70 |
| Women (%) | 56.2 | 56.4 | 50.0 | .61 |
| BMI[a] (kg/m$^2$) | 26.2 ± 3.6 | 26.2 ± .1 | 27.4 ± 1.2 | .19 |
| Waist/hip ratio | .90 ± .09 | .90 ± .03 | .94 ± .03 | .18 |
| Glucose (mmol/liter) | 7.6 ± 3.4 | 7.6 ± .1 | 10.7 ± 1.4 | .0008 |
| Fructoseamine (μmol/liter) | 318.8 ± 60.3 | 318.2 ± 22.5 | 342.4 ± 32.2 | .14 |
| Diabetes mellitus (%) | 24.4 | 22.3 | 56.3 | .02 |

NOTE.—All values are expressed as means with standard deviation (whole group) or means with standard errors (insulin-receptor variants).

[a] Body-mass index.

liter; $P = .20$) and fructosamine levels (422.7 vs. 356.7 μmol/liter; $P = .04$), indicating worse metabolic control.

## Discussion

A variety of insulin-receptor mutations has been related to insulin resistance, but only some of those mutations are also associated with glucose intolerance and manifest diabetes mellitus. The latter mutations are found predominantly in exons 17, 18, and 19 of the insulin-receptor gene, which encodes a part of the cytoplasmic region of the receptor (Taylor et al. 1992). We have confined ourselves to these exons during the search for mutations that associate with diabetes. The only mutant that emerged was the Val-Met mutation at position 985, bearing a relative risk of 4.49 for the presence of diabetes mellitus.

Position 985 is located in the juxtamembrane region of the insulin receptor, and this region represents an important structural domain for signal transduction and receptor internalization. The Tyr-residue at position 972 (or 960 in the numbering of the insulin receptor without exon 11) is critical for interaction of the insulin receptor with the signaling intermediates Shc and IRS1 (He et al. 1995; Eck et al. 1996). Although Val-985 is probably not directly involved in these interactions, mutations at position 985 may affect the interaction of the receptor with the signaling molecules IRS-1 and Shc by changing the spatial positioning of this part of the receptor. This possibility is corroborated by the recent observation that the Met-985 insulin-receptor variant behaves slightly differently in signaling (Strack et al. 1996). The juxtamembrane region also contains the Leu-Leu motif at positions 998–999, important for receptor internalization and degradation (Renfrew-Haft et al. 1994). Mutations in the vicinity of this motif, such as at 985, may affect receptor internalization and degradation. As a result, the signaling capacity of the receptor may be modulated.

Association studies are vulnerable to false-positive associations when the populations are not well matched for factors such as ethnicity, age, family relations, and poor diagnosis of the disease. The participants of the Rotterdam study were elderly individuals, aged >55 years, and >99% were of Dutch Caucasian origin (Hofman et al. 1991). All participants were examined using an OGTT, except for those with previously diagnosed diabetes. Diabetes mellitus was diagnosed on the basis of random and post-load serum glucose values, using the World Health Organization (WHO) criteria (WHO 1985).

It remains possible that some patients were misclassified, most likely as false-negative. If this occurred, however, it would lead to an overestimation of the Met-985 allele in the control group. The individuals found positive for the Met-985 allele did not have known relationships. Besides, they did not belong to an ethnic subgroup where diabetes mellitus is more prevalent. Finally, when two silent mutations in the insulin receptor are considered, a comparable frequency is seen in the diabetic and control group. On the basis of these data, we judge that a spurious genetic imbalance between the control and diabetic group is unlikely.

The Met-985 mutation itself may be the predisposing variant for hyperglycemia. Alternatively, this mutation could be in disequilibrium with another mutation that actually predisposes for hyperglycemia. The Met-985 receptor variant is not linked to silent polymorphisms in exon 3 and exon 17. Exon 3 is located at a site that could be as much as 100 kb away. The silent polymorphism at the His-1058 codon is in the same exon as Met-985 and only ~220 bp apart. The absence of linkage

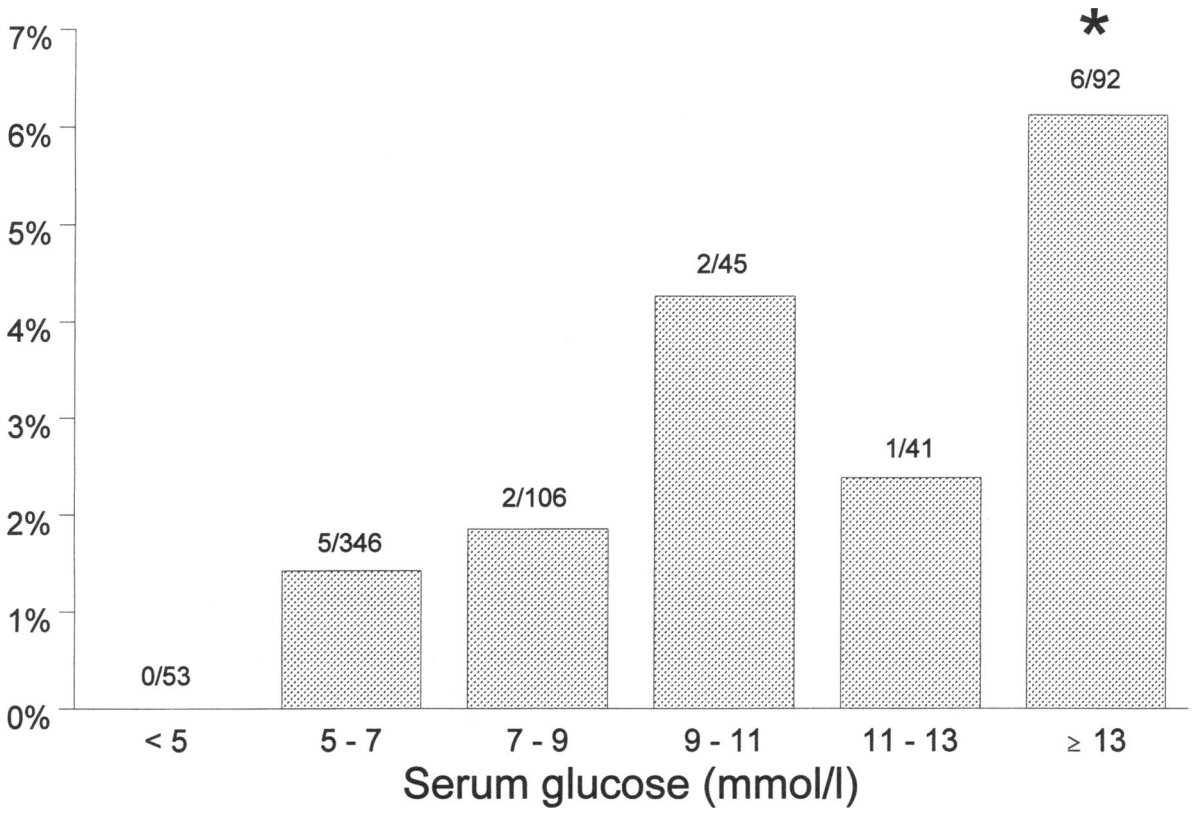


**Figure 3** The prevalence of the Met-985 allele, by categories of serum glucose. The numbers above each bar indicate subjects with a mutation and the total number of subjects in that category. An asterisk (*) indicates a test for trend: $P < .005$.

disequilibrium between the 985 and 1058 codons argues in favor of the hypothesis that the Met-985 allele is the predisposing variant for hyperglycemia. However, we cannot exclude that other mutations in nearby sequences, which do show linkage disequilibrium with the Met-985 allele, are the actual pathogenic mutations. The absence of linkage between the 985 and 1058 polymorphisms suggests the presence of the Met-985 allele during many generations or its frequent appearance as a de novo mutation.

We also examined DNA from 175 NIDDM patients and 88 healthy controls (not matched) from the Hoorn study (Nijpels et al. 1996) for the frequency of the Met-985 allele. The subjects in this group were aged >45 years and of Caucasian origin. All subjects in this study also underwent a OGTT to test their glucose tolerance.

Hoorn and Rotterdam are both cities in the western part of the Netherlands. Six diabetic individuals (3.4%) and one healthy control (1.1%) were heterozygous for the Met-985 allele, which is not significantly different from the frequencies found in the population-based Rotterdam study ($P > .20$).

The Met-985 allele was initially detected in insulin-resistant patients with lipodystrophy (O'Rahilly et al. 1991). When expressed in Chinese hamster ovary (CHO) cells, the Val and Met insulin-receptor variants both were functional (Flier et al. 1993). In vivo insulin-responsive tissues such as muscle and fat express insulin-responsive Glut4 glucose transporters, which are absent in CHO cells (Garvey and Birnbaum 1993). This situation hampers the extrapolation of data from transfected CHO cells to the activity of insulin-receptor variants in muscle and adipose tissue. Studies by Elbein et al. (1993) have provided some evidence that within pedigrees the Met-985 insulin-receptor variant associate with elevated postglucose load glucose levels. This observation is supported by the results from our study. No evidence for an association of the Met-985 allele with diabetes was previously found in a Welsh population (O'Rahilly et al. 1992). The number of participants in that study, however, was too small for detecting a significant association, in light of the low carrier frequency of Met-985 we found in subjects with diabetes mellitus. Another possibility is that the frequency of the Met-985 allele shows regional differences within Europe. Regional clustering of gene variants with diabetes has been observed in case of a mutation in the glucagon receptor (Hager et al. 1995; 't Hart et al. 1995).

In conclusion, our data indicate the association of the Met-985 insulin-receptor variant with hyperglycemia

and NIDDM in the Netherlands. The pathophysiological mechanism by which this mutation leads to glucose intolerance remains to be established, but it is expected that it contributes to the insulin-resistant component seen in NIDDM.

## Acknowledgments

We are indebted to the participants of the Rotterdam study and the research assistants of the research centers. This work was funded by the Netherlands Diabetes Fund and supported by the NESTOR program for geriatric research in the Netherlands (Ministry of Health and Ministry of Education).

## References

Beck-Nielsen H, Groop L (1988) Metabolic and genetic characterization of prediabetic states. Sequence of events leading to non-insulin-dependent diabetes mellitus. J Clin Invest 94: 1714–1721

Eck MJ, Dhe-Paganon S, Trüb T, Nolte RT, Shoelson SE (1996) Structure of the IRS-1 PTB domain bound to the juxtamembrane region of the insulin receptor. Cell 85:695–705

Elbein SC, Sorensen LK, Schumacher MC (1993) Methionine for valine substitution in exon 17 of the insulin receptor gene in a pedigree with familial NIDDM. Diabetes 42:429–434

Eriksson J, Fransilla-Kallunki A, Ekstrand A, Saloranta C, Widen E, Schalin C, Groop L (1989) Early metabolic defects in persons at increased risk for non-insulin-dependent diabetes mellitus. N Engl J Med 321:337–343

Feskens EJM, Loeber JG, Kromhout D (1994) Diet and physical activity as determinants of hyperinsulinemia: the Zutphen elderly study. Am J Epidemiol 140:350–360

Flier JS, Moller DE, Moses AC, O'Rahilly S, Chaiken RL, Grigorescu F, Elahi D, et al (1993) Insulin mediated pseudoacromegaly: clinical and biochemical characterization of a syndrome of selective insulin resistance. J Clin Endocrinol Metab 76:1533–1541

Garvey WT, Birnbaum MJ (1993) Cellular insulin action and insulin resistance. Ballieres Clin Endocrinol Metab 7:785–873

Hager J, Hansen C, Vaisse C, Vionnet N, Philippi A, Poller W, Velho G, et al (1995) A missense mutation in the glucagon receptor gene is associated with non-insulin-dependent diabetes mellitus. Nat Genet 9:299–304

Hattersley AT, Turner RC, Permutt MA, Patel P, Tanizawa Y, Chiu KC, O'Rahilly S, et al (1992) Linkage of type-2 diabetes to the glucokinase gene. Lancet 339:1307–1310

He W, O'Neill TJ, Gustafson TA (1995) Distinct modes of interaction of Shc and insulin receptor substrate-1 with the insulin receptor NPEY region via non-SH2 domains. J Biol Chem 270:23258–23262

Hofman A, Grobbee DE, Jong PTVM de, Ouweland FA van de (1991) Determinants of disease and disability in the elderly: the Rotterdam elderly study. Eur J Epidemiol 7:403–422

Imamura T, Takata Y, Sasaoka T, Takada Y, Morioka H, Haruta T, Sawa T, et al (1994) Two naturally occurring mutations in the kinase domain of insulin receptor accelerate degradation of the insulin receptor and impair the kinase activity. J Biol Chem 269:31019–31027

Krook A, Kumar S, Laing I, Boulton AJM, Wass JAH, O'Rahilly S (1994) Molecular scanning of the insulin receptor gene in syndromes of insulin resistance. Diabetes 43:357–368

Maassen JA, Kadowaki T (1996) Maternally inherited diabetes and deafness: a new diabetes subtype. Diabetologia 39: 375–382

Moller DE, Yokata A, Flier JS (1989) Normal insulin receptor cDNA sequence in Pima indians with NIDDM. Diabetes 88: 1496–1500

Nijpels G, Popp-Snijders C, Kostense PJ, Bouter LM, Heine RJ (1996) Fasting proinsulin and 2-h post-load glucose levels predict the conversion to NIDDM in subjects with impaired glucose tolerance: the Hoorn study. Diabetologia 39:113–119

O'Rahilly S, Choi WH, Patel P, Turner RC, Flier JS, Moller DE (1991) Detection of mutations in insulin-receptor gene in NIDDM patients by analysis of single-stranded conformation polymorphisms. 40:777–782

O'Rahilly S, Krook A, Morgan R, Rees A, Flier JS, Moller DE (1992) Insulin receptor and insulin-responsive glucose transporter (Glut4) mutations and polymorphisms in a welsh type 2 (non-insulin-dependent) diabetic population. Diabetologia 35:486–489

Renfrew-Haft CR, Klausner RD, Taylor SI (1994) Involvement of dileucine motifs in the internalization and degradation of the insulin receptor. J Biol Chem 269:26286–26294

Seino S, Seino M, Bell GI (1990) Human insulin receptor gene: partial sequence and amplifications of exons by polymerase chain reaction. Diabetes 39:123–128

Strack V, Bossenmaier B, Mushack J, Häring H-U (1996) 973 valine to methionine mutation of human insulin receptor (HIR): Functional characterization in 293 cells. Exp Clin Endocrinol Diabetes 104 (Suppl 2):100

Taylor SI, Cama A, Accili A, Barbetti F, Quon MJ, de la Luz Sierra M, Suzuki Y et al, (1992) Mutations in the insulin receptor gene. Endocrin Rev 13:566–595

't Hart LM, Stolk RP, Jansen JJ, Grobbee DE, Lemkes HHPJ, Maassen JA (1995) Absence of the Gly40-Ser mutation in the glucagon receptor among diabetic patients in the Netherlands. Diabetes Care 18:1400–1401

Vaag A, Henriksen JE, Beck-Nielsen H (1992) Decreased insulin activation of glycogen synthase in skeletal muscles in young non-obese Caucasian first degree relatives of patients with non-insulin-dependent diabetes mellitus. J Clin Invest 89:782–788

Van den Ouweland JMW, Lemkes HHPJ, Ruitenbeek W, Sandkuyl LA, de Vijlder MF, Struyvenberg PA, van de Kamp JJ, et al (1992) Mutation in mitochondrial tRNA (Leu,UUR) gene in a large pedigree with maternally transmitted type II diabetes mellitus and deafness. Nat Genet 1:368–371

Van der Vorm ER, Van der Zon GCM, Moller W, Krans HMJ, Lindhout D, Maassen JA (1992) An Arg for Gly substitution at position 31 in the insulin receptor, linked to insulin resistance, inhibits receptor processing and transport. J Biol Chem 267:66–71

Vionnet N, Stoffel M, Takeda J, Yasuda K, Bell GI, Zouali H, Lesage S, et al (1992) Nonsense mutations in the glucokinase gene causes early-onset non-insulin dependent diabetes mellitus. Nature 356:721–722

Warram JH, Martin BC, Krolewski AS, Soeldner JS, Kahn CR (1990) Slow glucose removal and hyperinsulinemia precede the development of type II diabetes in the offspring of diabetic parents. Ann Int Med 113:909–915

WHO (1985) Diabetes mellitus. Technical report series 727. World Health Organization, Geneva

Yki-Järvinen H (1994) Pathogenesis of non-insulin dependent diabetes mellitus. Lancet 343:91–95