Diabetologia (1996) 39: 1505–1511

Diabetologia
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UKPDS 19: Heterogeneity in NIDDM: separate contributions of IRS-1 and β3-adrenergic-receptor mutations to insulin resistance and obesity respectively with no evidence for glycogen synthase gene mutations

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Summary Insulin receptor substrate-1 (IRS-1), β3-adrenergic-receptor (β3-AR) and glycogen synthase (GS) genes are candidate genes for non-insulin-dependent diabetes mellitus (NIDDM), insulin resistance, dyslipidaemia and obesity. We studied white Caucasian subjects with NIDDM, 227 being randomly selected, 49 NIDDM within the top two percentiles of insulin resistance; 54 with dyslipidaemia in the top quintile of triglyceride/insulin and the bottom quintile of HDL, and 166 non-diabetic control subjects. We examined the association of the simple tandem repeat DNA polymorphisms (STRPs) near the IRS-1 and GS genes, and the prevalence of mutations at codons of IRS-1 513 and 972, β3-AR 64 and GS 464 using restriction fragment length polymorphism (RFLP). The STRP alleles in IRS-1 were significantly different between NIDDM and control subjects ($p = 0.015$). The IRS-1 972 mutation was significantly different between the four groups with increased prevalence in the insulin resistant and dyslipidaemia subjects (18 and 26 % compared with 11 % in control subjects; $p < 0.0005$). Those with or without IRS-1 mutations had similar clinical characteristics and impaired insulin sensitivity. β3-AR 64 mutation was not significantly different between the four groups but those with the mutation were more obese, with a test for linear association between number of alleles and degree of obesity in an analysis of variance showing a significant association ($p = 0.029$). The GS 464 mutation was not detected in any of the diabetic or control subjects and the population association study using GS STRP showed no difference in allelic frequencies between NIDDM patients and control subjects. A mutation in lipoprotein lipase at codon 291, associated in the general population with low HDL cholesterol, was not at increased prevalence in the NIDDM patients with dyslipidaemia. In conclusion, IRS-1 972 had an increased prevalence in subjects with insulin resistance, with or without dyslipidaemia. β3-AR 64 was associated with increased obesity but not with insulin resistance or dyslipidaemia. These separate contributions to different features of NIDDM are an example of the polygenic inheritance of this heterogeneous disorder. [Diabetologia (1996) 39: 1505–1511]

Keywords Insulin receptor substrate-1, β3-adrenergic-receptor, glycogen synthase, lipoprotein lipase, insulin resistance, dyslipidaemia.

Received: 9 April 1996 and in revised form: 5 August 1996

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Abbreviations: NIDDM, Non-insulin-dependent diabetes mellitus; IRS-1, insulin receptor substrate-1; GS, glycogen synthase; β3-AR, β3-adrenergic receptor; LPL, lipoprotein lipase; STRP, simple tandem repeat DNA polymorphism.

The common form of late-onset non-insulin-dependent diabetes mellitus (NIDDM) is a complex disorder in which at least two major defects contribute to the pathophysiology of the phenotype: insulin deficiency and insulin resistance [1–3]. It is likely that NIDDM is polygenic and that different phenotypes are due to a combination of different mutations in several genes [4–6] and to environmental influences [7, 8]. Candidate sites for genetic defects of insulin resistance include the insulin receptor, the insulin signal transduction pathway, and insulin-regulated genes

involved in peripheral utilization of glucose or suppression of hepatic glucose production [9]. Polymorphisms in the insulin receptor substrate-1 (IRS-1) [10–15], glycogen synthase (GS) [16, 17] and β3-adrenergic-receptor (β3-AR) [18–22] genes are candidate genes for insulin resistance and obesity, and have been reported to be associated with an increased risk for the development of NIDDM or obesity in Caucasians.

The major substrate of the insulin receptor tyrosine kinase has been identified as a 160–185 kDa phosphotyrosyl protein, IRS-1, which is believed to be the putative link in the insulin signalling pathway [23, 24]. It exhibits no intrinsic enzymatic activity but after phosphorylation it serves as a docking protein for binding other signal transduction molecules. Because IRS-1 has a key function in mediating the early steps in the action of insulin, it is a candidate for the site of the defect in insulin action seen in patients with NIDDM. Seven polymorphisms of IRS-1 have been studied and the mutation at the G972A (Gly to Arg) codon of IRS-1 is the most common polymorphism in all populations studied [15]. Also mutations at the G513C (Ala to Pro) and G972A codons of IRS-1 have been described that might be involved in the aetiology of a subset of NIDDM [10]. This has been corroborated in some studies [11–15] but not in others [25, 26]. An increased association of IRS-1 972 with obese subjects who had a low insulin sensitivity has raised the possibility of a genetic/environmental interaction [13].

Resistance to insulin in skeletal muscle has been attributed to concomitant obesity, particularly abdominal obesity and associated with glucose intolerance, hypertension, and dyslipidaemia [27–29]. Obesity is a known risk factor for the development of NIDDM [7, 8]. The β3-AR is expressed in visceral fat in humans [30] and is a regulator of lipolysis and the delivery of free fatty acids into the portal vein [31]. An increase in visceral fat mass, in turn, correlates with resistance to insulin in skeletal muscle [32]. An abnormality in the β3-AR could therefore explain the link between abdominal obesity and insulin resistance. Recent reports have shown that the Trp64Arg allele of the β3-AR is associated with abdominal obesity and resistance to insulin and may contribute to the early onset of NIDDM in Finnish, Pima and Japanese subjects [18–22].

GS is a key enzyme of the non-oxidative glucose disposal pathway in the skeletal muscle. Insulin resistance in the skeletal muscle is an early event in the development of NIDDM, which can exist before metabolic derangements occur [33, 34]. This suggests that molecular defects of the GS gene may contribute to the pathogenesis of NIDDM. Groop and co-workers [16] reported that the XbaI polymorphism of the GS gene was associated with development of insulin resistance and NIDDM in the Finnish population. A mutation G464A (Gly to Ser) was identified in two non-obese diabetic subjects [35] who had presented in the fourth decade and had dyslipidaemia with raised triglyceride and low HDL cholesterol.

Low HDL cholesterol levels are associated with decreased post-heparin activity of lipoprotein lipase [36, 37]. Reymer et al. [38] reported that decreased HDL-cholesterol in the serum can be associated with a specific lipoprotein lipase (LPL) mutation A291G (Asn to Ser) that was found significantly more often in patients with premature atherosclerosis. Whether this mutation could also contribute to the dyslipidaemia of patients with NIDDM is uncertain.

To determine whether the described mutations in the IRS-1, β3-AR and GS genes are associated with the development of NIDDM in British white Caucasian subjects, we studied their prevalence in a randomly selected NIDDM population and in two populations of NIDDM subjects in whom insulin resistance was a major feature, with and without dyslipidaemia, and in a control, non-diabetic population. We examined the prevalence of simple tandem repeat DNA polymorphisms (STRPs) near IRS-1 and GS genes in diabetic and control populations. We also assessed the prevalence of the LPL mutation in diabetic subjects with and without dyslipidaemia.

Subjects and methods

Subjects

Diabetic patients and non-diabetic subjects. We studied 227 unrelated British white Caucasian NIDDM subjects recruited into the Oxford clinic of the UK Prospective Diabetes Study (UKPDS) [39] and 166 non-diabetic white Caucasian subjects from the Oxford area who had fasting plasma glucose less than 6.0 mmol/l.

Diabetic patients with insulin resistance. We selected 49 NIDDM subjects with gross insulin resistance from the UKPDS. They were within the top two percentiles for raised fasting plasma insulin concentrations taking into account their obesity, by fitting a linear regression of plasma insulin against gender, age, and body mass index (BMI), and choosing those in the top two percentiles of residuals calculated from the model.

Diabetic patients with insulin resistance and dyslipidaemia. We selected 55 NIDDM subjects who had both insulin resistance and dyslipidaemia from the UKPDS. They had fasting plasma insulin and triglyceride levels in the top quintile and HDL levels in the bottom quintile of the population.

The phenotypes of the three NIDDM groups are summarised in Table 1. The biochemical methods have been described [40].

Genetic studies

The subjects were tested for the presence of the reported IRS-1 mutations at codons 513 and 972 and on a STRP of IRS-1, for

Table 1. Phenotypes of NIDDM subjects

	NIDDM		
	Random patients	Insulin resistance	Insulin resistance with dyslipidaemia
n	227	49	55
Age (years)	52 ± 9	51 ± 9	47 ± 9
BMI (kg/m^2)	29 ± 6	32 ± 6	33 ± 5
Fasting glucose (mmol/l)	12.5 ± 4.2	11.6 ± 3.5	12.0 ± 3.4
Fasting plasma insulin (mU/l)	14 (8–24)	37 (30–45)	28 (22–36)
Fasting plasma cholesterol (mmol/l)	5.6 ± 1.1	5.5 ± 1.0	6.0 ± 1.5
HDL cholesterol (mmol/l)	1.1 ± 0.2	1.0 ± 0.2	0.7 ± 0.1
triglyceride (mmol/l)	1.8 (1.0–3.2)	2.3 (1.4–3.7)	4.2 (2.7–6.5)
Beta-cell function (%β)	33 (16–70)	74 (43–128)	57 (32–101)
Insulin sensitivity (%S)	22 (13–37)	9 (7–11)	11 (9–14)

Mean ± SD or geometric mean (SD range) [48, 49]

mutations at β3-AR codon 64, GS codon 464 and a STRP of GS, and for mutation at LPL codon 291.

DNA was extracted from peripheral blood by using either the standard phenol/chloroform method or the Nucleon II DNA extraction kit (Scotlab, Glasgow, UK). Subjects were genotyped at STRPs near IRS-1 [41] and GS [42]; and for polymorphisms encompassing the codons of IRS-1 513 and 972 [10], β3 64, GS 464 and LPL 291, respectively using the polymerase chain reaction (PCR) (Perkin Elmer, Beaconsfield, UK). Primers used for the PCR are shown in Table 2. For the STRPs studies, the PCR products were separated by electrophoresis on a non-denaturing polyacrylamide gel. For the restriction fragment length polymorphism (RFLP) studies, restriction enzyme digestion was carried out using the appropriate restriction enzymes (Biolabs, Hitchin, UK) and the fragments were analysed after electrophoresis on a 3.5 % metaphor agarose gel. Positive controls for each codon were included in all experiments.

For IRS-1 513, three patterns were observed after DNA amplified by PCR was digested with DraIII: one band sized 268 bp for wild-type; three bands sized 268, 168 and 100 bp for Ala513/Pro513 heterozygous and two bands sized 168 bp, 100 bp for Pro513 homozygous. For IRS-1 972 codon, three patterns were observed after PCR product was digested with Bst NI: 3 bands sized 159, 81 and 23 bp for the wild-type; 5 bands sized 159, 108, 81, 51 and 23 bp for Gly972/Arg972 heterozygous and 4 bands sized 108, 81, 51 and 23 bp for Arg972 homozygous.

Digestion of the PCR product of β3-AR 64 with BstO1 produced fragments of the following sizes: 99, 62, 30, 12 and 7 bp for the wild-type; 161, 99, 62, 30, 12 and 7 bp in Trp64/Arg64 heterozygotes; and 161, 30, 12 and 7 bp in Arg64 homozygotes.

The mutation in the GS gene is located in exon 11 and it changes the codon 464 from Gly to Ser. This change (GGC to AGC) destroys a HaeIII restriction site. Two bands of the wild-type gene were observed after DNA was digested with HaeIII, 105 bp and 54 bp. Heterozygosity was characterised by three bands sized 159, 105 and 54 bp.

The LPL mutation, A 291G, which is located in exon 6, introduced an Rsa1 site that was recognised by 214 bp and 24 bp for heterozygosity fragments after RFLP in this study.

Statistical analysis

The allelic frequencies in the NIDDM and non-diabetic subjects were compared using chi-square or Fisher's exact test. Continuous variables were compared between the groups using analysis of variance, with log-transformed data when appropriate, and taking group stratification into account as a co-variate.

Results

Mutation screening

IRS-1 G972A mutation. Table 3 shows that 18 % of subjects with insulin resistance and 26 % with insulin resistance and dyslipidaemia had IRS-1 972

Table 2. Genetic markers used for the study of insulin receptor substrate 1, β3-adrenergic receptor, glycogen synthase and lipoproteinlipase

Localisation	Polymorphism/mutation	Primer sequence 5′ to 3′	Size of amplified fragment (bp)
Insulin receptor substrate-1	STRP	GTTCATTAATATTGTTCAACTGTGG AATTAATTTGAAACCCGTTTGATGG	132–140
	G513C	GCGGTGAGGAGGAGCTAA GCCACTGAGGACTGGGACGGG	268
	G972A	CTTCTGTCAGGTGTCCATCC TGGCGAGGTGTCCACGTAGC	263
β3-adrenergic receptor	T64A	CGCCCAATACCGCCAACAC CCACCAGGAGTCCCATCACC	210
Glycogen synthase	STRP	AGCTAATTTTTGTATCTGTG CCTGGGCATCAGAGCAAGAC	76–96
	G464A	TACCCTTCTTGTGGCTCCTG AGCCCTGACCAAATGCCCTC	159
Lipoprotein lipase	A291G	GCCGAGATACAATCTTGGTG CTGCTTCTTTTGGCTCTGACGTA	234

Table 3. Variants of insulin receptor substrate (IRS-1), β3- adrenergic receptor (AR) and glycogen synthase (GS) genes in the different subjects

Codon	Change	Cleavage enzyme	Random	NIDDM Insulin resistance	Dyslipidaemia	Control	*p* value (χ^2 or Fisher's exact test)
IRS-1 513	GCC → CCC (Ala → Pro)	DraIII	5/209	0/49	3/52	3/164	NS
IRS-1 972	GGG → AGG (Gly → Arg)	BstNI	14/208 (7 %)	9/49 (18 %)	14/54 (26 %)	18/164 (11 %)	$p < 0.0005$
β3-AR 64	TGG → CGG (Trp → Arg)	BstOI	34/209 (16 %)	11/46 (24 %)	6/50 (12 %)	18/152 (12 %)	NS
GSY 464	GGC → AGC (Gly → Ser)	HaeIII	0/211	0/49	0/55	0/164	

mutation, compared with 7 % of random diabetic subjects and 11 % of control subjects, with an increased prevalence in both insulin resistant dyslipidaemic and Dys groups (χ^2 $p < 0.0005$). None of the diabetic subjects were homozygous for the IRS-1 972 mutation.

IRS-1 G513C mutation. Table 3 shows no difference in prevalence between the groups.

Phenotype analysis showed no significant differences for any of the variables in Table 1 between the diabetic subjects with or without IRS-1 mutations.

β3-AR T64C mutation. The prevalence was not significantly different between the four groups (Table 3), but the diabetic subjects with the mutation were more obese, the five subjects with homozygous mutation had a BMI 35.6 ± 7.8 kg/m^2 (mean ± SD), the 46 with heterozygous mutation 31.1 ± 6.5 and the 254 with wild type being 29.7 ± 5.9 kg/m^2. A test for linear association between number of alleles and degree of obesity in an analysis of variance showed a significant association ($p = 0.029$). There was no difference for any of the other variables in Table 1 between those with and without the mutation. All the subjects with the homozygous mutation were diabetic.

GSG464A mutation. The mutation was not detected in any of the subjects.

LPL A291G mutation. There was no difference between the groups, with the mutation detected in 4 % of random NIDDM subjects, 3 % of NIDDM with insulin resistance plus dyslipidaemia and 3 % of control subjects. The subjects with the mutations had similar HDL concentrations to those without the mutation, in the random diabetic subjects mean 0.96 vs 1.04 mmol/l, and in dyslipidaemic subjects 0.65 vs 0.71 mmol/l.

Population association study. The allele frequencies for the STRPs of IRS-1 and GS in normal and diabetic subjects are shown in Table 4. There was no linkage disequilibrium for GS whereas the STRP alleles of IRS-1 were significantly different between NIDDM and control subjects ($p = 0.015$).

Discussion

The separate associations of the IRS-1 972 mutation in two groups of subjects who had insulin resistance, both with and without dyslipidaemia, and β3-AR codon 64 mutation only with obesity, suggested that they contribute to different aspects of NIDDM in a polygenic model. Neither was associated specifically with dyslipidaemia, which could also not be explained by the recently described LPL mutation associated with low HDL concentrations [38].

The association of the IRS-1 972 mutation with NIDDM in Danish white Caucasian NIDDM subjects [10] has been supported by some [11–15] but not all studies [25, 26]. Clausen et al. [13] suggested that IRS-1 codon 972 variant may interact with obesity to induce greater insulin resistance than would occur with either the mutation or obesity on its own. We found a significantly increased prevalence of IRS-1 972 mutation in subjects with NIDDM who had insulin resistance alone (18 %) in insulin resistance with dyslipidaemia (26 %) compared with 11 % in control subjects ($p < 0.0005$) but no increased prevalence in a random series of NIDDM subjects (7 %). The insulin resistance had been assessed as that greater than normally present in relation to the degree of obesity expressed as BMI, suggesting that IRS-1 972 contributed specifically to the marked insulin resistance present in some patients with NIDDM, with or without dyslipidaemia. There were no other phenotypic differences between those with or without the IRS-1 972 mutation. We found a significant difference for IRS-1 STRP between a random NIDDM and non-diabetic population which could not be explained by IRS-1 972, suggesting other IRS-1 promoter or gene mutations may contribute to insulin resistance.

Table 4. Allelic frequencies in diabetic and control subjects for insulin receptor substrate-1 and glycogen synthase STRPs

	Allele	Frequency in control subjects		Frequency in diabetic subjects	
		Proportion	n	Proportion	n
			n = 332		n = 454
Insulin receptor substrate-I	1	0.0783	26	0.0529	24
	2	0.5030	167	0.5925	269
	3	0.3404	113	0.3128	142
	4	0.0301	10	0.0264	12
	5	0.0482	16	0.0154	7
			n = 128		n = 334
Glycogen synthase	1	0.0391	5	0.0269	9
	2	0.0391	5	0.0449	15
	3	0.0469	6	0.0479	16
	4	0.0313	4	0.0090	3
	5	0.0938	12	0.1587	53
	6	0.3516	45	0.2814	94
	7	0.1172	15	0.1437	48
	8	0.0938	12	0.1048	35
	9	0.0938	12	0.1168	39
	10	0.0859	11	0.0449	15
	11	0.0078	1	0.0210	7

The finding that the missense mutation in β3-AR codon 64, particularly when homozygous, was associated with obesity in patients with NIDDM is in accord with studies in genetically obese mice and rats and clinical studies in man [43]. Obese Pima Indians with this mutation had an early onset of NIDDM and a tendency to have a low metabolic rate [19] and in Caucasians the mutation was associated with the early onset of NIDDM and clinical features of the insulin resistance syndrome (increased blood pressure and high serum concentrations of insulin, glucose and lipids) [18] but we found only an association with obesity. The subjects with the homozygous mutation were diabetic, which is in keeping with other data, although on its own the homozygous mutation is not sufficient to induce diabetes, although it can present at a younger age [22]. We did not find a specific association with insulin resistance or dyslipidaemia suggesting that the mutation may contribute to obesity that can lead to diabetes, but otherwise does not affect the type of diabetes that occurs.

GS is a candidate gene for insulin resistance since insulin-stimulated GS activity is reduced in skeletal muscle of patients with NIDDM and their first-degree relatives and reduced GS mRNA in skeletal muscle has been reported [33, 34, 44]. The possibility of mutations in GS causing insulin resistance was suggested by the report that the A_2 allele of the XbaI polymorphism of the GS gene can be associated with insulin resistance in subjects with NIDDM in some reports [33, 45] but not all studies [17, 46]. A GS mutation (codon G464A Gly to Ser) was found in two diabetic patients with abnormal lipids in Finland [35], but we did not detect this mutation in any of the random NIDDM subjects or in those with insulin resistance or dyslipidaemia or in control subjects. As we studied insulin-resistant diabetic subjects with dyslipidaemia similar to the reported G464A phenotype, it is unlikely that it is commonly associated with diabetes in white Caucasians. We also found no difference in the prevalence of alleles of GS STRP between the NIDDM and control subjects.

Mutations in LPL usually produce the specific syndrome of pregnancy-induced chylomicronaemia [47], but a recently discovered mutation in codon 291 was associated with low HDL cholesterol in patients with premature atherosclerosis in the general population [38]. We found no association of this mutation with diabetes or dyslipidaemia. Thus, the reported mutations that we studied in IRS-1, β3-AR or LPL do not account for the dyslipidaemia of NIDDM.

Specific relationships between mutations and pathophysiologies can be studied by identifying patients with mutations in a random diabetic population and assessing associations; examples are IRS-1 972 [13] and β3-AR 64 in this and previous studies [18, 19]. The additional examination of groups of patients with NIDDM, with and without specific features, can be helpful in defining the genetic contributions to phenotypes.

Subjects with NIDDM have varying degrees of obesity, insulin resistance, dyslipidaemia and hyperglycaemia. While environmental and developmental factors are involved, the specific association of an IRS-1 mutation with insulin resistance and of the β3-AR mutation with obesity is likely to presage identification of mutations in many genes, that will contribute to the marked variability in phenotype in this heterogeneous disorder.

Acknowledgements. We are grateful to Professor O. Pedersen for DNA with IRS-1 mutations for positive controls. This study was supported by grants from the Wellcome Trust and the Alan

and Babette Sainsbury Trust. We thank Ms. I. Samuel and Ms. C. Wood for their assistance with the manuscript.

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