

# Searching for type 2 diabetes genes: prospects in pharmacotherapy

G Sesti

Department of Experimental and Clinical Medicine, University of Catanzaro–'Magna Graecia', Catanzaro, Italy

*The Pharmacogenomics Journal* (2002) 2, 25–29. DOI: 10.1038/sj/tpj/6500078

Type 2 diabetes is a complex, heterogeneous group of metabolic disorders that has reached epidemic proportions, affecting over 5% of the population in western countries. The pathophysiology of type 2 diabetes includes two apparently distinct defects, ie impairments in insulin action at the level of skeletal muscle, fat and liver, and a failure in insulin secretory capacity of pancreatic  $\beta$ -cells. There is strong evidence that genetic factors play an important role in both of these components given the familial nature, the high concordance rate between monozygotic twins, and the high prevalence in certain ethnic groups.<sup>1</sup> However, identifying genes that confer susceptibility to type 2 diabetes has proven problematic. The search for diabetes-predisposing genes has mainly relied on two complementary approaches: (1) the candidate genes analysis; and (2) the positional cloning using genome-wide linkage analysis. A number of genes have been screened as putative candidates and several polymorphisms in coding and promoter regions have been tested in case-control association studies. So far, only one gene predisposing to the common form of type 2 diabetes has been identified by positional cloning,<sup>1</sup> while this approach was successfully employed to identify genes responsible for rare monogenic forms of diabetes such as maturity-onset diabetes of the young (MODY).<sup>2–7</sup> Here, I will review results of studies of 'functional' and 'positional' candidate genes predisposing to type 2 diabetes, focusing

on those that may influence the variability in patients' response to antidiabetic drugs or may be amenable to pharmacological intervention.

## CANDIDATE GENE STUDIES

Although considerable effort has been devoted to identify genes that contribute to diabetes susceptibility, the genetic basis of common forms of type 2 diabetes has not yet been identified. The study of candidate genes is one of the most popular approaches to this question. A candidate gene is defined as a gene whose protein product has important functions in insulin secretion, insulin action, and/or adipose metabolism. Although abnormalities in insulin receptor activation appear to be the earliest defects in most patients with type 2 diabetes, mutations in the insulin receptor gene have been only described in rare syndromes of severe insulin resistance such as type A insulin resistance, Leprechaunism, and Rabson–Mendenhall.<sup>8</sup> However, these mutations typically behave in a recessive manner and cause severe insulin resistance only if they are present in the homozygous or in the compound heterozygous state. Although it is estimated that 1–2% of patients with common forms of type 2 diabetes are heterozygous carriers, it is unlikely that mutations in the insulin receptor gene are responsible for more than a small fraction of the variation in insulin sensitivity observed in the diabetic population. More than 60 potential candidate genes have been screened in the search for type 2 susceptibility genes and several common variants have been identified in many of these genes (Table 1).

## Gene Involved in Adipogenesis

The common Pro12Ala variant in the peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ), a nuclear hormone receptor that regulates adipogenesis by acting as a heterodimer with retinoid X receptor (RXR), has been associated with a decreased risk for type 2 diabetes in several studies.<sup>9,10</sup> The Pro12-Ala variant showed *in vitro* a modest decrease in both binding affinity to the cognate promoter element and ability to transactivate responsive promoters, which may account for the lower body mass index and improved insulin sensitivity observed in individuals carrying the variant.<sup>9</sup> Accordingly, heterozygous knockout mice of PPAR $\gamma^{+/-}$  showed increased insulin sensitivity and appear to be protected from high fat diet-induced adipocyte hypertrophy, obesity, and insulin resistance.<sup>11</sup> These findings may seem at odds with the antidiabetic effects of thiazolidinediones, a new class of antidiabetic drugs that improves insulin sensitivity by acting through PPAR $\gamma$  receptors. However, recent studies have attempted to explain the mechanisms whereby both pharmacological activation of PPAR $\gamma$  by thiazolidinediones and moderate reduction in PPAR $\gamma$  activity can lead to amelioration in insulin resistance.<sup>12,13</sup> The results of these studies have led to the hypothesis that pharmacological activation of PPAR $\gamma$  markedly increases triglyceride content of adipose tissue, thereby decreasing triglyceride content of skeletal muscle and liver, leading to improvement of insulin sensitivity at the expense of body weight gain due to increased adipose tissue mass.<sup>12</sup> These changes were accompanied by stimulation of adipocyte differentiation and apoptosis, thereby increasing the number of small adipocytes, which eventually lead to amelioration of insulin sensitivity presumably via a decrease in molecules causing insulin resistance such as free fatty acids and tumor necrosis factor  $\alpha$  (TNF $\alpha$ ).<sup>12</sup> Moderate reduction in PPAR $\gamma$  activity as

**Table 1 Common variants of candidate genes for type 2 diabetes**

Insulin -23 A/T (HphI)	HNF-1 $\alpha$ Ala98Val
Insulin receptor Val 985Met	HNF-4 $\alpha$ Val225Met
IRS-1 Gly972Arg	HNF-4 $\alpha$ Thr130Ile
IRS-1 Ala512Pro	FABP2 Ala54Thr
IRS-2 Gly1057Asp	$\beta$ 2 adrenergic receptor Gln27Glu
IRS-2 Leu647Val	$\beta$ 3 adrenergic receptor Trp64Arg
p85 subunit PI3-kinase Met326Ile	UCP2 Ala55Val
Shc Met300Val	UCP2 -866 G $\rightarrow$ A
PC1 Lys121Gln	UCP3 -55 C $\rightarrow$ T
GLUT4 Val383Ile	TNF $\alpha$ -238 A/G
PPAR $\gamma$ Pro12Ala	Paraoxonase-2 Ala148Gly
SUR1 C/T (exon 18) Thr759Thr	Hexokinase II Gln142Pro
SUR1 IVS15-3 c/t	Glycogen synthase Met416Val
KIR 6.2/Bir Glu23Lys	Glycogen-associated regulatory subunit of type 1 protein phosphatase (PPP1R3)
Glucagon receptor Gly40Ser	Asp905Tyr
Amylin Ser20Gly	Calpain 10 G/A intron 3 (UCSNP-43)
Islet amyloid polypeptide -132 G $\rightarrow$ A	
PDX-1 Asp76Asn	

observed in PPAR $\gamma^{+/-}$  mice, or partial impairment in its function, as seen in carriers of the Pro12Ala variant, would ameliorate insulin sensitivity by decreasing triglyceride content of adipose tissue, skeletal muscle, and liver due to a combination of increased leptin expression, reduced expression of lipogenic enzymes, increased fatty acid combustion and energy dissipation.<sup>12</sup> These changes were accompanied by reduction in body weight due to decreased adipose tissue mass, which results in a decrease in free fatty acids and TNF $\alpha$ .<sup>12</sup> The data showing that moderate reduction in PPAR $\gamma$  activity resulted in amelioration of insulin sensitivity have been further confirmed by the observation that treatment of mice with RXR or PPAR $\gamma$  antagonists results in changes similar to those observed in PPAR $\gamma^{+/-}$  mice leading to protection against high fat diet-induced obesity and associated insulin resistance.<sup>13</sup> However, other explanations may account for the increased insulin sensitivity observed in PPAR $\gamma^{+/-}$  mice including differential interaction of co-activators or repressors with PPAR $\gamma$  receptors in the heterozygote state. The identification of a novel role for PPAR $\gamma$  in obesity and insulin resistance associated with type 2 diabetes may inspire novel therapeutic strategies including both PPAR $\gamma$  agonists and antagonists for obesity and obesity-linked type 2 diabetes.

#### Genes Involved in $\beta$ -cell Function

Another common polymorphism that has been shown to decrease protein function is the Gly972Arg change in the insulin receptor substrate 1 (IRS-1).<sup>14-18</sup> Both diabetic patients and glucose-tolerant subjects carrying the Gly-972Arg IRS-1 variant are characterized by impaired insulin secretion.<sup>17</sup> In addition, human pancreatic islets isolated from carriers of this variant and rat  $\beta$ -cell line transfected with and expressing Gly972Arg IRS-1 exhibited defects in binding of the p85 subunit of PI 3-kinase to IRS-1 and IRS-1 associated PI 3-kinase activity.<sup>15,18</sup> These changes caused a marked decrease in insulin secretion in response to glucose or sulfonylurea.<sup>15</sup> Two variants, a synonymous ACC $\rightarrow$ ACT substitution in the exon 18, and a c $\rightarrow$ t intron variant in position -3 of the exon 16 splice acceptor site (IVS15-3c $\rightarrow$ t), in the high affinity sulfonylurea receptor (SUR1) gene, have been reported to be associated with type 2 diabetes.<sup>19</sup> Interestingly, carriers of both the exon 18 C/T or T/T and the IVS15-3c $\rightarrow$ t c/t or t/t genotypes are characterized by lower insulin secretion upon tolbutamide injection.<sup>20</sup> Although the two common variants in SUR1 and IRS-1 have been variably associated with type 2 diabetes and their pathogenetic role in secondary failure to sulphonylurea therapy has not been directly addressed, *in vivo* and *in vitro* evidence suggests that these two single nucleotide

polymorphisms may represent potential examples of pharmacogenomics in type 2 diabetes. In spite of intense investigation, no single major disease-predisposing mutations in candidate genes have been thus far identified. Studies on candidate genes often take the form of association studies, which can be carried out in either related or unrelated subjects. Population stratification, ie the existence of more than one ancestral source of a population's gene pool, represents a major limitation of this study design. In addition, it is likely that failure of candidate gene analysis to detect diabetes-predisposing genes results from a low prior probability of the involvement of the few gene polymorphisms examined in the overall risk of the disease.<sup>21</sup> Other limitations of candidate gene studies are related to modest sample size that can fail to detect true associations as well as to lack of replication in different populations due to genetic background factors.<sup>21</sup> Moreover, the search for genetic defects that contribute to the development of type 2 diabetes has proven to be problematic due to the fact that: (1) it is genetically heterogeneous, ie in a given population not all individuals with diabetes have the same genetic defects; (2) it is polygenic with complex inheritance patterns, ie a single individual exhibits a disorder due to simultaneous inheritance of two or more defects; and (3) interaction between genetic predisposition and environmental factors such as diet, physical activity, and age are required to result in overt diabetes. Thus, mutations or polymorphisms causing only modest defects in protein function, such as the Pro12Ala PPAR $\gamma$  or the Gly972Arg IRS-1 variants could significantly impair glucose homeostasis when coupled to other genetic or environmental factors.

#### Genes Involved in Thermogenesis

Another question that may complicate the search for a susceptibility gene for type 2 diabetes is that a certain gene could have tissue-specific divergent effects on glucose homeostasis. This issue has been raised from recent studies on the metabolic role of uncoupled

ling protein-2 (UCP2), a member of the mitochondrial inner membrane carrier family that is highly expressed in adipose tissue and pancreatic islets.<sup>22,23</sup> Like the homologous prototype UCP1, UCP2 mediates a mitochondrial proton leak, releasing energy stored within the proton-motive force as heat which, ultimately, results in a decrease in ATP production. Because an increase in ATP promotes insulin secretion in pancreatic  $\beta$ -cells, UCP2 could be a negative regulator of insulin secretion. In support of this view, UCP2 knockout mice showed higher serum insulin and C-peptide levels, and improved glucose tolerance.<sup>22</sup> In addition, pancreatic islets from UCP2 knockout mice exhibited increased ATP levels and increased insulin secretion.<sup>22</sup> Expression of UCP2 was increased in pancreatic islets of *ob/ob* mice, a model of obesity-induced diabetes. More importantly, crossing *ob/ob* mice with UCP2 knockout mice resulted in *ob/ob* mice lacking UCP2 that, although still severely obese, showed amelioration in insulin secretion and glucose tolerance.<sup>22</sup> Thus, an increased expression or activity of UCP2 in pancreatic  $\beta$ -cells may contribute to impair insulin secretion in type 2 diabetes. In contrast with these results, it has been reported that the common -866 G→A polymorphism in the promoter of the human UCP2 gene, which enhances its transcriptional activity, is associated with increased mRNA levels in human fat cells and a reduced risk of obesity, the major risk factor for type 2 diabetes.<sup>23</sup> These data have led to the hypothesis that an increase in UCP2 levels in adipocytes of carriers of the -866 G→A polymorphism may have beneficial effects on adiposity by increasing energy expenditure. However, recent studies have shown that the activity of UCP2 was lower than that of UCP1 and its role as uncoupling protein has been confuted.<sup>24</sup> The results of these studies raise the possibility that potential pharmacological agents for type 2 diabetes, such as inhibitors of UCP2 activity, may have beneficial effects on a specific tissue, ie pancreatic islets, but detrimental effects on another one, ie adipose

tissue. It thus may be necessary to design tissue-specific approaches in order to achieve a decrease in UCP2 in pancreatic  $\beta$ -cells that leads to an increase in insulin secretion, and a simultaneous increase in UCP2 in fat to promote weight loss and energy expenditure in subjects with type 2 diabetes and obesity.

#### Genes Involved in $\beta$ -cell Function of MODY

Some rare forms of diabetes result from mutations in a single gene. Among these, maturity-onset diabetes of the young (MODY) is an autosomal dominant disease accounting for approximately 2% of all diabetes patients; thus far, six forms of MODY have been identified, each involving mutations in genes that play an important role in  $\beta$ -cell function. These genes include hepatic nuclear factor-4 $\alpha$  (HNF-4 $\alpha$ , MODY1), glucokinase (associated with MODY2), HNF-1 $\alpha$  (MODY3), insulin promoter factor-1 (IPF-1, MODY4), HNF-1 $\beta$  (MODY5) and NUROD1/BETA2 (MODY6).<sup>2-7</sup> It has been recently reported that MODY3 patients with HNF-1 $\alpha$  mutations exhibit altered insulin secretory responses to glucose<sup>25</sup> but are more sensitive to sulfonylureas than type 2 diabetic patients.<sup>26,27</sup> Although the molecular mechanism underlying this hypersensitivity to sulfonylureas is unknown, treatment of MODY3 patients with sulfonylureas, which is the commonest therapy in all forms of MODY, should be cautiously introduced due to the considerable risk of hypoglycemia.

#### GENOME-WIDE LINKAGE ANALYSIS

Genome-wide linkage analysis is another traditional approach that has been employed to identify susceptibility genes for type 2 diabetes. This approach allows regions of chromosomal DNA to be identified that are shared (linkage) among affected family members to an extent that would not be expected by chance alone. The increasing availability of large numbers of highly informative genetic markers, including microsatellite markers composed of short nucleotide

repeats, and, more recently, single nucleotide polymorphisms (SNPs), has allowed whole genome screening to identify chromosomal regions that are linked with type 2 diabetes by genotyping family members. A gene located in a chromosomal region that has been found to be linked to type 2 diabetes is referred to as a positional candidate. A gene whose encoded protein might not be considered itself as a candidate by virtue of its function may suddenly become a plausible candidate by virtue of its presence in a linked region.

#### Calpain 10, the First Positional Candidate Gene

Linkage studies in Mexican-American families localized a susceptibility locus for type 2 diabetes, termed *NIDDM1*, on chromosome 2q37.3.<sup>28</sup> This locus appears to act in concert with another one on chromosome 15 to increase susceptibility to diabetes in American Mexicans.<sup>29</sup> The gene encoding calpain-10, a nonlysosomal cysteine protease, was positionally cloned within the *NIDDM1* region.<sup>30</sup> One SNP (UCSNP-43), a G→A transition within intron 3 of the calpain 10 gene, was associated with diabetes and evidence for linkage. Among Pima Indians, a population with a very high incidence and prevalence of type 2 diabetes, G/G homozygous carriers were characterized by higher fasting glucose, and 2-h insulin levels, and decreased rates of glucose disposal and oxidation.<sup>31</sup> However, G/G homozygosity at UCSNP-43 was not associated with a significantly increased risk of diabetes, but rather specific haplotypes, which include UCSNP-43 in combination with two other polymorphisms within the calpain 10 gene.<sup>30</sup> The calpains are a superfamily of calcium-activated neutral proteases that are widely distributed in tissues. Calpains have been implicated in a variety of cellular processes including apoptosis, proliferation, differentiation, and may regulate intracellular signalling, adipocytes differentiation,<sup>32</sup> as well as insulin-induced downregulation of IRS-1.<sup>33</sup> Calpain 10 is expressed in three important tissues responsible for glucose production (liver), glucose uptake

(skeletal muscle), and insulin production (pancreatic  $\beta$ -cells),<sup>30</sup> each of which is functionally defective in type 2 diabetes. Recent *in vitro* studies have shown that nonspecific calpain inhibitors increase the insulin secretory response to glucose in mouse pancreatic islets, but reduce insulin-mediated glucose transport in isolated rat muscle strips and adipocytes.<sup>34</sup> Thus, calpain 10 may be considered as a component of a new pathway involved in insulin action and, possibly, insulin secretion that may include substrates, inhibitors and activators, each of which could be amenable to pharmacological intervention. Interestingly, another gene of the calpain family, the calpain 3 gene, is located in the region of chromosome 15 containing the susceptibility locus that interacts with *NIDDM1* to increase susceptibility to diabetes in American Mexicans.<sup>29</sup>

#### Other Diabetes-Susceptibility Chromosomal Loci

The results of genome scans for type 2 diabetes have indicated the presence of diabetes-susceptibility loci on chromosome 1q in Pima Indians<sup>35</sup> and Caucasians,<sup>36</sup> on chromosome 11q in Pima Indians, also linked to BMI,<sup>35</sup> on chromosomes 3,4,9 and 10 in Mexican Americans,<sup>37</sup> and on chromosomes 12q and 20q in Finnish.<sup>38,39</sup>

Another susceptibility locus for type 2 diabetes has been mapped to chromosome 3q27, where the adiponectin (also known as Acrp30 and AdipoQ) gene is located.<sup>40</sup> Two further loci on chromosomes 3 and 14, respectively, have been linked to plasma adiponectin concentrations.<sup>41</sup> Adiponectin is one of the proteins secreted by adipose tissue, referred to as 'adipokines', which include also leptin, TNF $\alpha$ , adipisin and resistin. Although the pathophysiological role of these adipokines remains to be firmly established, several reports indicate that they play an important role in fat metabolism, feeding behavior, energy balance and insulin sensitivity. Recent studies have shown that adiponectin expression is reduced in omental and subcutaneous adipose tissue of obese type 2 diabetic patients.<sup>42</sup> Plasma adiponectin con-

centrations are reduced in obese and type 2 diabetic subjects and are correlated to insulin resistance.<sup>43,44</sup> More importantly, administration of adiponectin to animal models of obesity-induced diabetes results in a decrease in glucose, free fatty acid and triglyceride plasma levels, amelioration of insulin resistance, and reduction in triglyceride content in muscle and liver.<sup>45,46</sup> The site of action of adiponectin remains controversial. Some authors suggest that it acts by enhancing the anti-gluconeogenic effects of insulin in liver<sup>46</sup> while others suggest that adiponectin increases fatty acid oxidation and energy dissipation in muscle, leading to a decrease in tissue triglyceride content, free fatty acid and triglyceride plasma levels.<sup>45</sup> Although these results are preliminary and uncertainty remains regarding the mechanism of action of adiponectin, these studies raise the intriguing possibility that adiponectin or its synthetic analogs might represent a novel pharmacological approach to type 2 diabetes.

#### CONCLUSIONS

The model for the progression of type 2 diabetes is characterized by an initial state of insulin resistance leading to increased  $\beta$ -cell insulin secretion with compensatory hyperinsulinemia. As long as hyperinsulinemia is adequate to overcome insulin resistance, glucose tolerance remains normal. When the  $\beta$ -cell fails to compensate, insulin secretion cannot keep pace with the underlying insulin resistance and glucose intolerance and, eventually, frank diabetes occurs. This model suggests a number of potential points where one might intervene to manage or even possibly to prevent type 2 diabetes. These include the resolution or reduction of insulin resistance, treatment of  $\beta$ -cell failure or both. A major advantage of the Human Genome Project is that it will allow the identification of more 'functional' or 'positional' candidate genes for type 2 diabetes and will allow the structural and functional characterization of even the most elusive of human genes. In addition, the genome project will help to find new potential pharmaco-

logical targets including enzymes, receptors, substrates and transcription factors whose function is directly or indirectly related to type 2 diabetes. But identifying true disease-related genes on the basis of their putative role in insulin action and secretion may be misleading, as illustrated by the unexpected role of calpain 10 as susceptibility gene for type 2 diabetes.<sup>30</sup> As the molecular mechanism regulating insulin secretion and insulin action unfolds, we will have increasing possibilities to elucidate the role of specific signaling elements in insulin action and  $\beta$ -cell function, and to design efficacious, safe and convenient pharmacological interventions.

#### DUALITY OF INTEREST

None declared.

#### Correspondence should be addressed to

G Sesti, MD, Dipartimento di Medicina Sperimentale e Clinica, Università di Catanzaro-'Magna Græcia', Via Tommaso Campanella, 115, 88100 Catanzaro, Italy.  
Tel: 011 39 0961 772626  
Fax: 011 39 0961 772626  
E-mail: sestig@unicz.it

#### REFERENCES

- 1 Permutt MA, Hattersley A. Searching for type 2 diabetes genes in the post genome era. *Trends Endocrinol Metab* 2000; **11**: 383–393.
- 2 Yamagata K, Furuta H, Oda N, Kaisaki PJ, Menzel S, Cox NJ *et al*. Mutations in the hepatocyte nuclear factor-4 $\alpha$  gene in maturity-onset diabetes of the young. *Nature* 1996; **384**: 458–460.
- 3 Vionnet N, Stoffel M, Takeda J, Yasuda K, Bell GI, Zouali H *et al*. Nonsense mutation in the glucokinase gene causes early-onset non-insulin-dependent diabetes mellitus. *Nature* 1992; **356**: 721–722.
- 4 Yamagata K, Oda N, Kaisaki PJ, Menzel S, Furuta H, Vaxillaire M *et al*. Mutations in the hepatocyte nuclear factor-1 $\alpha$  gene in maturity-onset diabetes of the young. *Nature* 1996; **384**: 455–458.
- 5 Stoffers DA, Ferrer J, Habener JF. Early-onset-type-II diabetes mellitus (MODY4) linked to IPF1. *Nat Genet* 1997; **15**: 106–110.
- 6 Horikawa Y, Iwasaki N, Hara M, Furuta H, Hinokio Y, Cockburn BN *et al*. Mutation in hepatocyte nuclear factor-1 $\beta$  gene (TCF2) associated with MODY. *Nat Genet* 1997; **17**: 384–385.
- 7 Malecki MT, Jhala US, Antonellis A, Fields L, Doria A, Orban T *et al*. Mutations in NEURODI are associated with the development of type 2 diabetes mellitus. *Nat Genet* 1999; **23**: 323–328.

- 8 Taylor SI, Cama A, Accili D, Barbetti F, Quon MJ, de la Luz Sierra M *et al.* Mutations in the insulin receptor gene. *Endocrine Rev* 1992; **13**: 566–595.
- 9 Deeb SS, Fajas L, Nemoto M, Pihlajamaki J, Mykkanen L, Kuusisto J *et al.* A Pro12Ala substitution in PPAR $\gamma$ 2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. *Nat Genet* 1998; **20**: 284–287.
- 10 Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J *et al.* The common PPAR $\gamma$  Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet* 2000; **26**: 76–80.
- 11 Kubota N, Terauchi Y, Miki H, Tamemoto H, Yamauchi T, Komeda K *et al.* PPAR $\gamma$  mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. *Mol Cell* 1999; **4**: 597–609.
- 12 Yamauchi T, Kamon J, Waki H, Murakami K, Motojima K, Komeda K *et al.* The mechanisms by which both heterozygous PPAR $\gamma$  deficiency and PPAR $\gamma$  agonist improve insulin resistance. *J Biol Chem* 2001; **276**: 41245–41254.
- 13 Yamauchi T, Waki H, Kamon J, Murakami K, Motojima K, Komeda K *et al.* Inhibition of RXR and PPAR $\gamma$  ameliorates diet-induced obesity and type 2 diabetes. *J Clin Invest* 2001; **108**: 1001–1013.
- 14 Almind K, Inoue G, Pedersen O, Kahn CR. A common amino acid polymorphism in insulin receptor substrate-1 causes impaired insulin signaling. *J Clin Invest* 1996; **97**: 2569–2575.
- 15 Porzio O, Federici M, Hribal ML, Lauro D, Accili D, Lauro R *et al.* The Gly<sup>972</sup>->Arg amino acid polymorphism in IRS-1 impairs insulin secretion in pancreatic  $\beta$ -cells. *J Clin Invest* 1999; **104**: 357–364.
- 16 Hribal ML, Federici M, Porzio O, Lauro D, Borboni P, Accili D *et al.* The Gly->Arg<sup>972</sup> amino acid polymorphism in IRS-1 affects glucose metabolism in skeletal muscle cells. *J Clin Endocrinol Metab* 2000; **85**: 2004–2013.
- 17 Stumvoll M, Fritsche A, Volk A, Stefan N, Madaus A, Maeker E *et al.* The Gly972Arg polymorphism in the insulin receptor substrate-1 gene contributes to the variation in insulin secretion in normal glucose-tolerant humans. *Diabetes* 2001; **50**: 882–885.
- 18 Federici M, Hribal ML, Ranalli M, Marselli L, Porzio O, Lauro D *et al.* The common Arg<sup>972</sup> polymorphism in insulin receptor substrate-1 causes apoptosis of human pancreatic islets. *FASEB J* 2001; **15**: 22–24.
- 19 Inoue H, Ferrer J, Welling CM, Elbein SC, Hoffman M, Mayorga R *et al.* Sequence variants in the mayoyoga receptor (SUR) gene are associated with NIDDM in Caucasians. *Diabetes* 1996; **45**: 825–831.
- 20 Hansen T, Echwald SM, Hansen L, Moller AM, Almind K, Clausen JO *et al.* Decreased tolbutamide-stimulated insulin secretion in healthy subjects with sequence variants in the high-affinity sulfonylurea receptor gene. *Diabetes* 1998; **47**: 598–605.
- 21 Risch NJ. Searching for genetic determinants in the new millennium. *Nature* 2000; **405**: 847–856.
- 22 Zhang CY, Baffy G, Perret P, Krauss S, Peroni O, Grujic D *et al.* Uncoupling protein-2 negatively regulates insulin secretion and is a major link between obesity,  $\beta$  cell dysfunction, and type 2 diabetes. *Cell* 2001; **105**: 745–755.
- 23 Esterbauer H, Schneitler C, Oberkofler H, Ebenbichler C, Paulweber B, Sandhofer F *et al.* A common polymorphism in the promoter of UCP2 is associated with decreased risk of obesity in middle-aged humans. *Nat Genet* 2001; **28**: 178–183.
- 24 Stuart JA, Harper JA, Brindle KM, Jakabsons MB, Brand MD. Physiological levels of mammalian uncoupling protein 2 do not uncouple yeast mitochondria. *J Biol Chem* 2001; **276**: 18633–18639.
- 25 Byrne MM, Sturis J, Menzel S, Yamagata K, Fajans SS, Dronsfield MJ *et al.* Altered insulin secretory responses to glucose in diabetic and nondiabetic subjects with mutations in the diabetes susceptibility gene MODY3 on chromosome 12. *Diabetes* 1996; **45**: 1503–1510.
- 26 Sovik O, Njolstad P, Folling I, Sagen J, Cockburn BN, Bell GI. Hyperexcitability to sulphonylurea in MODY3. *Diabetologia* 1998; **41**: 607–608.
- 27 Pearson ER, Liddell WG, Shepherd M, Corrall RJ, Hattersley AT. Sensitivity to sulphonylureas in patients with hepatocyte nuclear factor-1 alpha gene mutations: evidence for pharmacogenetics in diabetes. *Diabet Med* 2000; **17**: 543–545.
- 28 Hanis CL, Boerwinkle E, Chakraborty R, Ellsworth DL, Concannon P, Stirling B *et al.* A genome-wide search for human non-insulin-dependent (type 2) diabetes genes reveals a major susceptibility locus on chromosome 2. *Nat Genet* 1996; **13**: 161–166.
- 29 Cox NJ, Frigge M, Nicolae DL, Concannon P, Hanis CL, Bell GI *et al.* Loci on chromosome 2 (NIDDM1) and 15 interact to increase susceptibility to diabetes in Mexican Americans. *Nat Genet* 1999; **21**: 213–215.
- 30 Horikawa Y, Oda N, Cox NJ, Li X, Ortho-Melander M, Hara M *et al.* Genetic variations in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. *Nat Genet* 2000; **26**: 163–175.
- 31 Baier LJ, Permana PA, Yang X, Pratley RE, Hanson RL, Shen G-Q *et al.* A calpain-10 gene polymorphism is associated with reduced muscle mRNA levels and insulin resistance. *J Clin Invest* 2000; **106**: R69–R73.
- 32 Patel YM, Lane DM. Role of calpain in adipocytes differentiation. *Proc Natl Acad Sci USA* 1999; **96**: 1279–1285.
- 33 Smith LK, Rice KM, Garner CW. The insulin-induced down-regulation of IRS-1 in 3T3-L1 adipocytes is mediated by a calcium-dependent thiol protease. *Mol Cell Endocrinol* 1996; **122**: 81–92.
- 34 Sreenan SK, Zhou Y-P, Otani K, Hansen PA, Currie KPM, Pan C-Y *et al.* Calpains play a role in insulin secretion and action. *Diabetes* 2001; **50**: 2013–2020.
- 35 Hanson RL, Ehm MG, Pettitt DJ, Prochazka M, Thompson DB, Timberlake D *et al.* An autosomal genomic scan for loci linked to type II diabetes mellitus and body-mass index in Pima Indians. *Am J Hum Genet* 1998; **63**: 1130–1138.
- 36 Wiltshire S, Hattersley AT, Hitman GA, Walker M, Levy JC, Sampson M *et al.* A genome-wide scan for loci predisposing to type 2 diabetes in a UK population (the Diabetes UK Warren 2 repository): analysis of 573 pedigrees provides independent replication of a susceptibility locus on chromosome 1q. *Am J Hum Genet* 2001; **69**: 553–569.
- 37 Duggirala R, Blangero J, Almasy L, Dyer TD, Williams KL, Leach RJ *et al.* Linkage of type 2 diabetes mellitus and of age at onset to a genetic location on chromosome 10 q in Mexican Americans. *Am J Hum Genet* 1999; **64**: 1127–1140.
- 38 Mahtani MM, Wide'n E, Lehto M, Thomas J, McCarthy M, Brayer J *et al.* Mapping of a gene for NIDDM associated with an insulin secretion defect by a genome scan in Finnish families. *Nat Genet* 1996; **14**: 90–95.
- 39 Ghosh S, Watanabe RM, Hauser ER, Valle T, Magnuson VL, Erdos MR *et al.* Type 2 diabetes: evidence for linkage on chromosome 20 in 716 Finnish affected sib pairs. *Proc Natl Acad Sci USA* 1999; **96**: 2198–2203.
- 40 Vionnet N, Hani El-H, Dupont S, Gallina S, Francke S, Dotte S *et al.* Genome-wide search for type 2 diabetes-susceptibility genes in French whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21-q24. *Am J Hum Genet* 2000; **67**: 1470–1480.
- 41 Comuzzie AG, Funahashi T, Sonnenberg G, Martin LJ, Jacob HJ, Black AE *et al.* The genetic basis of plasma variation in adiponectin, a global endophenotype for obesity and the metabolic syndrome. *J Clin Endocrinol Metab* 2001; **86**: 4321–4325.
- 42 Statnick MA, Beavers LS, Conner LJ, Corominola H, Johnson D, Hammond CD *et al.* Decreased expression of apM1 in omental and subcutaneous adipose tissue of humans with type 2 diabetes. *Int J Exp Diabetes Res* 2000; **1**: 81–88.
- 43 Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J *et al.* Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 1999; **257**: 79–83.
- 44 Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE *et al.* Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab* 2001; **86**: 1930–1935.
- 45 Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K *et al.* The fat-derived hormone adiponectin reverses insulin resistance associated with both lipotrophy and obesity. *Nature Med* 2001; **7**: 941–946.
- 46 Berg AH, Combs TP, Du X, Brownlee M, Scherer PE. The adipocyte-secreted protein Acrp 30 enhances hepatic insulin action. *Nature Med* 2001; **7**: 947–953.