# 4. GENETIC ASPECTS OF DIABETES MELLITUS

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## 1.1. Molecular Basis of Diabetes Mellitus

Diabetes mellitus is a heterogeneous group of disorders in which particular disease phenotypes can be characterized by a specific aetiology and/or pathogenesis of the disease; in many cases their aetiologic and pathogenetic classification is greatly impeded due to significant phenotype overlapping.

## 1.2. Genetic Basis of Type 1 Diabetes Mellitus

Type 1 diabetes mellitus (DM) or insulin-dependent diabetes mellitus (IDDM), which is characterized by absolute insulin deficiency, is the most common form of diabetes in children and the young population. It results primarily from a lesion of the pancreatic b-cell. The pathogenesis of type 1 diabetes includes genetic predisposition for the disease and environmental factors that are able to activate the mechanisms which lead to a progressive loss of pancreatic b-cells (Fig. 1). Type 1 diabetes is a multi-factorial autoimmune disorder showing familial aggregation, the rate of familial aggregation being consistent with the significance of the genetic contribution to the disease.

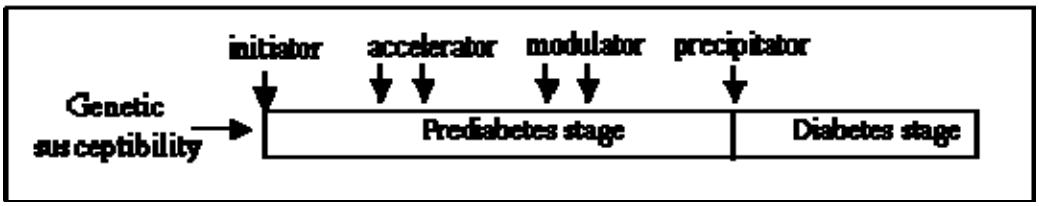


**Figure 1.** *Schematic presentation of the sequence of events that may lead to type 1 diabetes in a genetically susceptible individual*

The genetic aetiology of type 1 diabetes involves genes of the human leukocyte antigen (HLA) system and so-called non-HLA genes. Results of many studies have shown the risk of the development of diabetes to be 30% - 65% in monozygotic twins, and about 20% in non-twin HLA-identical pairs. Irrespective of genetic compatibility, the disease prevalence among first generation relatives ranges between 6% and 10%, while in the general population it is 0.4% (Table 1). Accordingly, HLA genes play an important role in predisposing to type 1 diabetes. Non-HLA genes, however, also contribute to this particular susceptibility. At least 17 chromosomal regions have been associated with this susceptibility to diabetes, suggesting that type 1 diabetes is a polygenic disorder (Table 2). A comprehensive understanding of this genetic interplay will arise from analysis of the structure and function of candidate genes, providing an insight into the mechanism of the disease via HLA system molecules and from elucidating the route of the disease that crosses with the genetic contribution of non-HLA genes.

Table 1. Empirical risk for type 1 diabetes

| | % |
|---|---|
| Monozygotic twins | 30 – 70 |
| HLA-identical siblings | 15 |
| Average risk in siblings | 6 – 10 |
| General population | 0.4 |

Table 2. HLA-DQ molecule susceptibility to type 1 diabetes

| DQ molecule | Population | Haplotype | Relative risk |
|---|---|---|---|
| **Positive link** | | | |
| A1*0301-B1*0302 | Multiple | DRB1*04-DQB1*0302 (cis) | 2.4 – 9.5 |
| A1*0501-B1*0201 | Multiple | DRB1*301-DQB1*0201 (cis) | 2.8 – 5.1 |
| A1*0501-B1*0302 | Multiple | DRB1*04-DQB1*0302/ DRB1*301-DQB1*0201 (trans) | 17.1– 32.0 |
| A1*0301-B1*0201 | Multiple | DRB1*04-DQB1*0302/ DRB1*301-DQB1*0201 (trans) | |
| A1*0301-B1*0402 | Caucasians | DRB1*04-DQB1*0302/ DRB1*801-DQB1*0402 (trans) | 4.01 – 16.0 |
| A1*0301-B1*0401 | Japanese | DRB1*04-DQB1*0401 (cis) | 3.4 – 4.4 |
| A1*0301-B1*0201 | Blacks | DRB1*701-DQB1*0201 (cis) | 8.4 – 12.9 |
| A1*0301-B1*0303 | Japanese | DRB1*901-DQB1*0303 (cis) | 2.2 – 4.62 |
| A1*0301-B1*0201 | Blacks | DRB1*901-DQB1*0201 (cis) | 5.5 |
| **Negative link** | | | |
| A1*0102-B1*0602 | Multiple | DRB1*15001-DQB1*0602 (cis) | 0.03 – 0.2 |

| | | | |
|---|---|---|---|
| A1*0103-B1*0603 | Multiple | DR 1301-DQB1*0603 (cis) | 0.04 – 0.2 |
| A1*0301-B1*0301 | Multiple | DRB1*04-DQB1*0301 (cis) | 0.18 – 0.5 |
| A1*0501-B1*0301 | Multiple | DRB1*1101-DQB1*0301 (cis) | 0.041- 0.4 |

### 1.2.1. HLA system genes

HLA system genes, extending over 3.7 kb DNA, are located on the short arm of chromosome 6. Within the HLA region, they are grouped into three classes. Class 1 genes (HLA-A, HLA-B and HLA-C) encode class 1 HLA antigens, located on the surface of all nucleated cells, where they represent endogenously formed peptides on CD8+ cytotoxic T cells. Class 2 genes (HLA-DR, HLA-DQ and HLA-DP) produce class 2 HLA antigens that are found exclusively on B-lymphocytes, macrophages, epithelial cells of the islets of Langerhans, and activated T lymphocytes, i.e. in the monocyte-macrophage cell line. Their expression on other cells may be induced by cytokines such as g-interferon and TNF-a, which also may contribute to immune recognition and autoimmunity. Several genes different from DR, DQ and DP loci have also been characterized in HLA class 2 between the HLA DQ and DP loci. Class 3 genes code for complement components (C2, properdin factor B, C4A and C4B), 21-hydroxylase and products involved in T cell-mediated inflammation, such as TNF-A and TNF-B, and acute phase protein.

HLA genes are characterized by pronounced polymorphism, which leads to their high functional diversity in immunoregulation. For example, each class 2 locus has 20 – 100 different alleles in a population, which differ among themselves by 1% - 15% allele sequences.

The main HLA locus of genes susceptible to type 1 diabetes is found within the HLA region on the short arm of chromosome 6. The HLA region was first related to diabetes when its association with several class I HLA antigens (HLA-B8, -B18 and -B15) was detected by serotyping. The results of subsequent studies have shown the class II HLA antigens to be even more closely associated with the disease than class I HLA antigens. So, a great majority of patients were found to have class II HLA-DR3 or HLA-DR4 antigens, and some 30% of patients to have a DR3/DR4 heterozygous genotype. The DR3/DR4 heterozygous genotype has been associated with the highest risk of developing type 1 diabetes with a synergistic effect, immediately following the homozygous DR4 and DR3 genotypes. It also seems that DR4 gene can verify such a susceptibility as a dominant trait, whereas DR3 may be expressing a recessive trait. Based on DNA sequencing, the HLA-DQ locus has been found to be much more strongly associated with diabetes than the DR locus. The alleles of this locus code for a number of HLA-DQ antigen types, heterodimers, which consist of two glycoprotein chains (a and b), involved in immuno-recognition and antigen presentation. As differentiated from the HLA-DR a-chain, the locus DQ a- and b-chains are highly polymorphic.

In Caucasians, the HLA-DQ heterodimers (a-chains are designated as DQA1, and b-chains as DQB1) encoded by DQA1*0301, DQB1*0302 and DQA1*0501, DQB1*0201 alleles, are most strongly associated with diabetes and provide a link with HLA-DR4 and DR3 alleles in disbal-

ance. Transcomplementation of the four polymorphic DQ a and b chains from opposite haplotypes significantly enhances variations in the two antigens involved in the immune response, which has been proposed as an explanation for the increased risk of diabetes observed in DR3, QA1*0501, DQB*0201/DR4,DQA1*0301,DQB1*0302 heterozygotes. The more so, allelic variations on the DQB1 locus separate two of the most common HLA-DR4 loci found in Caucasians, based on the presence of the DQB1*0302 or the DQB1*0301 alleles. Compared with the general population, a majority (95%) of DR4 haplotypes found in diabetic patients carry DQB1*0302 allele. The independent effect of DQB1*0201 allele cannot be demonstrated because of the high linkage disbalance between DQB1*0201 and DRB1*0301 alleles in Caucasians with DR3 haplotype. Moreover, the DQB1*0201 allele does not confirm the increased susceptibility to diabetes when linked to the DRB1*0701 allele on DR7 haplotype. Different levels of the risk for diabetes allocated to the DQB1*0201 allele on DR3 and DR7 haplotypes could be explained by different DQA1 alleles (*501 on DR3 and 0201 on DR7), which are linked to the DQB1*0201 allele on these haplotypes.

It has also been observed that DQB1*0302 differs from DQB1*0301 at position 57, where it lacks the asparagine residue. The DQB1*0201 allele also lacks asparagine at position 57, thus it is considered that this amino acid residue might be involved in the molecular mechanism of encoded susceptibility to type 1 diabetes. In fact, it appears that the amino-acid residue at position 57 of DQ-b chain is of critical importance for peptide binding and recognition. It also seems that other DQ-b chain amino-acid residues might influence the susceptibility to diabetes. The amino acid residue substitutions at positions 57 and 70 have been observed to correlate strongly with the risk of diabetes development. An arginine residue at position 52 of the DQ-a chain also correlates with diabetes susceptibility.

However, some DQB1 low risk genotypes, including DQB1*0302/DQB1*0201 (DR7), DQB1*0201 (DR3)/DQB1*0201 (DR3) and DQB1*0201 (DR3)/DQB1*0201 (DR7), also lack asparagine at position 57.

Irrespective of the contribution of individual amino acid residues at position 57, the coded susceptibility for type 1 diabetes seems mostly to be consequential to the HLA-DQ locus allele in the class II region. However, many studies have shown that DRB1 alleles also modulate the susceptibility to diabetes, and that the HLA class III region might also be involved. TNF gene appears to be a serious candidate from class III of the HLA system, since this gene polymorphism can induce the production of tumour necrosis factor a (TNF-a), which is a potent cytokine, thus affecting the immune response potential.

### 1.2.2. Protective effect of class 2 HLA genes

Certain haplotypes of class 2 HLA genes exert a protective action against the development of diabetes. In a majority of the population, HLA-DR2 haplotypes show negative correlation with type 1 diabetes and act as protection from the risk of its development, even in heterozygotes carrying the disease-susceptible DR4-DQB1*0302 haplotype. However, the protection elicited by these alleles is not absolute in patients carrying the DQB1*0602 allele. There is evidence for the role of the DQB1*0602 allele in protection from the development of diabetes in individuals in whom the unusual DR2 haplotype lacking the DQB1*0602 allele has been detected. Other haplotypes that negatively correlate with type 1 diabetes, e.g.,

DR5 haplotypes in blacks, have also been occasionally described. In Caucasians, DR5 is linked with DQB1*0301 and DQA1*0501 alleles, whereas in blacks DR5 haplotypes carry DQB1*0201 and DQA1*0301 alleles predisposing to diabetes. Also, among DR6 haplotypes, DRB1*1301-DQA1*0103-DQB1*0603 alleles may be associated with a decreased susceptibility for the development of the disease.

### 1.2.3. Use of HLA genetic markers in identifying the risk of type 1 diabetes

At present, there is strong evidence for the association between HLA system genes and susceptibility for type 1 diabetes. Molecular analysis of HLA genes has allowed for a more detailed definition of candidate genes. However, characterization of the priorities in their association with the disease is still impossible due to the strong linkage disbalance among HLA system haplotypes. A combination of the association of haploid links and cross-matched racial studies has confirmed the significant role of DQB1 in the susceptibility to type 1 diabetes. In Caucasians, 70% - 95% of patients are carriers of the DQB1 allele, a dominantly prone allele, whereas DR3-DQB1*0201 is a less predisposing genetic factor which, however, implies an increased risk in patients with the cis/trans DQB1*0302 genotype. The formation of trans heterodimers, which include DQB1 and DQA1 genes, may account for the increased risk associated with the patient's heterozygosity. DQbAsp57 has been demonstrated to be a critical residue in antigen binding, but cannot be added to the genetic complexity associated with type 1 diabetes. It seems that class 2 genes are possible, but not absolute, genes the mutations of which can lead to type 1 diabetes. To date, no specific genes responsible for the development of type 1 diabetes have been identified, as the genesis of the disease is consequential to a complex impact of a variety genetic and environmental predisposing factors. It appears, however, that the genetic protection, associated with specific HLA haplotypes, predominates over susceptibility. The protective effect of the DR2-DQB1*0602 haplotype may have interesting implications for the mechanisms of immunoregulation of the immune events associated with diabetes.

### 1.2.4. Non-HLA system genes

Studies investigating the association of non-HLA system genes with the development of diabetes have led to the detection of a number of candidate genes associated with the disease (Table 3.3). These include the genes encoding for the complex of T-cell receptors (TCR), manganese superoxide dismutase (MnSOD), interferon (IFN)-g, immunoglobulin and interleukin 1 (IL-1) system molecules, insulin, and tumour necrosis factor (TNF). On almost all genes, numerous polymorphisms have been found, the occurrence of which has been associated with diabetes. However, additional studies are needed to answer the question whether the functional consequences of these polymorphisms related to gene modifications are mediated via the immune system or by direct action on the pancreatic b-cells.

Table 3. Type 1 diabetes susceptible loci identified by linkage analysis

| Locus | Chromosome | Candidate genes or microsatellite markers |
|---|---|---|
| IDDM1 | 6p21.3 | HLA |
| IDDM2 | 11p15.5 | INS-VNTR |
| IDDM3 | 15q26 | D15S107 |

| | | |
|---|---|---|
| IDDM4 | 11q13.3 | FDF3, D11S1917, MDUI, ZFM1, RT6, ICE, CD3, LRP5, FADD |
| IDDM5 | 6q25 | ESR, a046Xa9, MnSOD |
| IDDM6 | 18q12-q21 | D18S487, D18S64, JK (kidd locus) |
| IDDM7 | 2q31-33 | D2S152 |
| IDDM8 | 6q25-27 | D6S281, D6S264, D6S446 |
| IDDM9 | 3q21-25 | D3s1303 |
| IDDM10 | 10p11-q11 | D10S193, D10S208 |
| IDDM11 | 14q24.3-q31 | D14S67 |
| IDDM12 | 2q33 | CTLA-4, CD28 |
| IDDM13 | 2q34 | D2S137, D2S164, IGFBP2, IGFBP5 |
| IDDM14 | undetermined | undetermined |
| IDDM15 | 6q21 | D6S283, D6S1580 |

### 1.3. Genetic Basis of Type 2 Diabetes Mellitus

Type 2 diabetes mellitus or non-insulin dependent diabetes mellitus (NIDDM) is a heterogeneous multifactorial syndrome characterized by abnormality in insulin action (resistance) and irregular insulin secretion (b-cell lesion). Type 2 diabetes includes subtypes, which are strongly associated with environmental and genetic factors. Aetiologically, it is of utmost importance to differentiate the genes that cause type 2 diabetes from those that contribute (predispose) to the onset of the disease. These two gene categories have different characteristics and require different methodologies for their detection.

Among monogenic forms of type 2 diabetes, the maturity-onset diabetes of the young (MODY), characterized by the onset at a young age (in childhood, adolescence or young adult age) as an autosomal dominant trait, has been most extensively studied over the last few years. Various phenotypes in MODY patients suggest the disorder to be genetically heterogeneous (Table 3.3). Now, MODY is known to include a number of different monogenic entities that differ clinically and metabolically. MODY 1 diabetes is caused by mutation of the nuclear factor-4a gene (HNF-4a/MODY1), MODY 2 by mutation of the glucokinase gene (GCK/MODY2), and MODY 3 by mutation of the hepatocyte nuclear factor-1a gene (HNF-1a/MODY3). About 20% of the families with MODY are not related to these three loci, indicating that at least one or more additional loci should be detected and identified in the near future (Table 4).

Table 4. Comparison of MODY* subtypes

| | MODY 1 | Glucokinase (MODY 2) | MODY 3 | non-MODY 1, 2 or 3 |
|---|---|---|---|---|
| *Chromosomal location* | 20q | 7p | 12q | unknown |
| *Onset of hyperglycaemia* | adolescence early adult age | early childhood (since birth?) | adolescence early adult age | uncertain |
| *Severity of hyperglycaemia* | progressive, may transform to extremely severe | mild, with minor exacerbation with age | progressive, may transform to extremely severe | varying |
| *Microvascular complications* | common | rare | common | varying |
| *Pathophysiology* | β-cell dysfunction | β-cell dysfunction | β-cell dysfunction | β-cell dysfunction |
| *Impaired glucose sensitivity* | NO | YES | NO | unknown |
| *Response to hyperglycaemia* | not present | present | present in normo-glycaemics | unknown |

*maturity-onset diabetes of the young (MODY)

Although the prevalence of MODY is not completely known, it appears to be much higher than anticipated, as it has been occurring all over the world, proceeding unrecognized almost to adult age. Reports from India indicate that MODY might account for as much as 18% of type 2 diabetes cases diagnosed 35 years ago. More than 10% of all forms of diabetes mellitus in young American blacks from the southeast of the USA refer to MODY. Also, MODY was recorded in 13% of Caucasian families with diabetes in France.

The genetic variation of MODY is related to its clinical and metabolic heterogeneity. MODY 2 is characterized by mild hyperglycaemia, decreased hepatic glycogen deposition, and enhanced postprandial gluconeogenesis. MODY 1 and MODY 3 are characterized by severe disturbance of insulin secretion and severe hyperglycaemia associated with microvascular complications.

### 1.3.1. MODY 1

MODY 1 is a type 2 diabetes caused by mutation of the hepatic nuclear factor-a (HNF-4a/MODY) gene located on the long arm of chromosome 20. Clinically, the MODY 1 phenotype indicates that these patients have suffered b-cell lesion, this even before the detection of hyperglycaemia, and may be characterized by a reduced maximal insulin secretion, which is disproportionate to the concentration of glucose.

### 1.3.2. MODY 2 – Glucokinase mutation

Glucokinase (GK) mutations are the most common cause of MODY identified to date. So far, 42 mutations of this gene have been detected in individuals of various races and ethnicity, including Caucasians from Brazil, France, Italy, Sweden, Switzerland, Great Britain and USA, Asians from Japan, blacks from the Congo, and non-mixed populations such as American Africans and Porto Ricans. Its 28 mutations change the protein sequence by single amino acid substitution; six mutations transform the sequence from one mRNA position to another, resulting in abnormal RNA; eight mutations are responsible for the incomplete protein synthesis by the formation of premature termination codon, either by point mutation or by deletion. Most of the mutations described to date were detected in a single family each, suggesting that such mutations will certainly be continually found in the future.

Glucokinase is produced in the pancreas and liver, and belongs to the family of hexokinases that catalyse phosphorylation of glucose-6-phosphate. It plays the main role in the regulation and integration of glucose metabolism in pancreatic b-cells and hepatocytes. In pancreatic b-cells, the metabolism of glucose and the secretion of insulin are strongly dependent on enzymatic activity, whereas in the liver glucokinase is included in the metabolism of postprandial glucose.

Studies have shown that mis-sense mutations have a varying effect on the activity of glucokinase, ranging from minor modification in the affinity for glucose through complete inactivity. Observations in models of human glucokinase in b-cells have shown that the known mis-sense mutations can be divided into three main categories: mutations of the preserved active site residue, which drastically affect the catalytic activity; mutations that compromise enzyme structure and reduce its activity; and mutations on the surface of the amino acid residue which eliminate interactions with other residues, and thus may impair stability of the structure or induce conformational changes. These mutations show only a minor activity reduction.

Clinical phenotype – patients with glucokinase mutations have a similar phenotype with moderate fasting hyperglycaemia (6-9 mmol/L) persisting from early childhood. Diabetic complications, especially microvascular ones, are very rare in these patients, and they can usually be successfully managed by diet alone. Pregnant women are an exception to the rule, as they generally require insulin to maintain normoglycaemia. Impaired b-cell function has been found in individuals with deficient glucokinase. This b-cells lesion is responsible for their insensitivity for glucose.

### 1.3.3. MODY 3

In 1995, Vaxillaire et al. described a new locus for MODY, located on the long arm of chromosome 12, which is likely to be the major cause of MODY. This gene, designated as MODY 3, has been identified as a gene coding for the hepatocyte nuclear factor 1a (HNF 1a) and liver transcription factor. MODY 3 develops consequentially to mutation of the hepatocyte nuclear factor-1a (HNF-1a/MODY3) gene located on chromosome 12q.

Clinical phenotype – MODY 3 phenotype differs from the glucokinase phenotype. The pa-

tients inheriting the gene are firstly euglycaemic and develop diabetes in adolescence or early adult age. Hyperglycaemia is progressive and requires intensive treatment.

Microvascular complications (retinopathy) are quite common. A non-obese patient presenting with symptomatic hyperglycaemia in adolescence or early adulthood may be misdiagnosed as having insulin-dependent diabetes mellitus. Physiological studies have shown that these patients suffer a b-cell lesion, which may precede the onset of diabetes and which assumes a considerably more severe form than in patients with deficient glucokinase.

### 1.3.4. Mitochondrial mutations

Mitochondria are unique intracellular organelles that possess their own DNA. Mitochondrial DNA (mtDNA) consists of 16,529 bases encoding for 13 enzymatic subunits included in oxidative phosphorylation, 12 transport RNA and 12 ribosomal RNA. Until 1992, the role of mitochondrial mutations in diabetes was not considered highly significant. Mutations, deletions and duplications of mtDNA were first described in mitochondrial myopathy. The occurrence of diabetes and glucose intolerance was observed in some of these patients. The occurrence of diabetes in the absence of myopathy but associated with mtDNA lesion was recorded on examining a large number of families with diabetes and hearing loss due to a hereditary factor.

Clinical phenotype associated with A3243G mtDNA mutation – hereditary diabetes mellitus and hearing loss associated with mutation at position 3243 mtDNA occur in 0.3% - 3% of diabetic patients. The disease affects all races, however, the highest percentage of these patients has been recorded in the Japanese. Diabetes primarily occurs due to the impaired function of pancreatic b-cells, however, most patients develop type 2 diabetes. The onset of the disease usually occurs in the third and fourth decade of life, but other age groups may also be affected (age 10-80). The 3243 mtDNA mutation has been associated with progressive neural hearing loss of a varying degree. Other pathologic states associated with this mutation include pigmentary retinopathy, cardiomyopathy and nephropathy.

### 1.3.5. Insulin receptors

The significant role of insulin resistance in type 2 diabetes has led to a concept according to which insulin receptors are considered as an important candidate gene for development of type 2 diabetes. Studies in non-obese patients with high insulin resistance have just pointed to mutations of the insulin receptor gene. All the mutations described are found in the structural part of the gene, most of them representing a single nucleotide substitution. The mutations exert a varying effect on the receptor expression and function, leading to a decreased level of the receptor mRNA, impaired proreceptor processing to a and b subunits, impaired receptor transport to cell membranes, precipitated receptor breakdown, abnormal insulin binding, and deranged activity of tyrosine kinase.

Most patients with a severe form of insulin resistance syndrome have homozygous or associated heterozygous mutations. Heterozygous mutations in the domain of tyrosine kinase can lead to severe insulin resistance. This predominant adverse effect seems to result from insulin receptor which, as a hybrid with mutant b-chain and wild type b-chain, is inactive to

tyrosine kinase. More than 40 different mutations of the insulin receptor gene have been discovered to date.

Clinical phenotypes – insulin receptor mutations lead to a variety of clinical syndromes:

- Type A insulin resistance is characterized by hyperandrogenism and severe insulin resistance in non-obese patients or patients with lipoatrophy;
- Rabson-Mendelhal syndrome is characterized by the features of type A insulin resistance, dysmorphic features, dental dysplasia, and pineal hyperplasia;
- Donohue's syndrome is characterized by intrauterine growth deficiency, fasting hypoglycaemia, hyperthyroidism, some features of type A insulin resistance, and limited life expectancy.

### 1.3.6. Insulin gene

Insulin gene mutations do not represent a major cause of diabetes mellitus, but were among the first diabetes-inducing mutations described. These mutations result in the inactive form of insulin molecule (proinsulin) being unable to undergo the process of cleavage to insulin.

Clinical phenotype – like insulin receptor mutations, these patients also have a discrete type 2 diabetes-like phenotype. Insulin gene mutation results in an extreme increase of serum insulin or proinsulin level, usually with a normal level of C-peptide and normal exogenous insulin sensitivity.

## 1.4. Genes Predictive of Type 2 Diabetes Mellitus

Genes predictive of type 2 diabetes have been defined on the basis of studies in families with diabetes mellitus by use of non-parametric linkage analysis and population studies. This group of genes include the genes encoding for insulin, insulin receptor mutation (IRS 1), HLA, FABP-2, glycogen synthase, glucagon receptor, and b-adrenergic receptor. However, insufficient evidence has been collected to date to confirm any of the above mentioned genes as being sensitive or specific enough for the early detection of diabetes mellitus.

### 1.4.1. Insulin resistance

Insulin resistance is a diminished ability of insulin to perform its biological function. Although individuals with insulin resistance secrete abnormally high amounts of insulin to compensate for the disturbance, to stimulate glucose transport to the muscular and adipose tissue, and to inhibit the hepatic production of glucose, the plasma concentration of glucose is on a continuous rise. In western countries, insulin resistance is widely spread and associated with various abnormalities, including obesity, hypertension, hyperlipidaemia and hyperuricaemia, as well as a predominantly sedentary lifestyle.

Many molecular disturbances are associated with insulin resistance. These include decreased expression of insulin receptors on the insulin-responsible cell surface, impairment of the transmission signal activated upon glucose binding to the receptor, and abnormalities of the metabolic pathways that are normally stimulated by insulin, including glucose trans-

port and glycogen synthesis. Insulin receptor gene mutations are responsible for insulin resistance in a limited number of individuals. However, in most type 2 diabetic patients the molecular basis of insulin resistance remains unknown. Although insulin resistance is an important factor for the development of type 2 diabetes, a majority of people with insulin resistance do not develop diabetes; some of them – although eventually turning diabetic – may have had insulin resistance for years without any substantial increase in their plasma glucose concentration. Physiological variations between the individuals with insulin resistance who develop diabetes and those who do not are highly important for the understanding of the pathophysiology of type 2 diabetes mellitus. As b-cells are able to produce more insulin to compensate for insulin resistance and to maintain normoglycaemia, the pancreatic b-cells are obviously the site where other causes of the disease should be searched for.

### 1.4.2. Obesity

Obesity is a metabolic disturbance showing the spread of an worldwide epidemic, and denoting an imbalance between energy intake and energy consumption. The state of obesity is associated with dyslipidaemia, hyperinsulinaemia, insulin resistance and impaired glucose tolerance. It is a risk factor for diabetes, hypertension, cardiac disease, other diseases, and increased mortality rate. The so-called obesity gene (ob) or leptin gene, and its product leptin have been identified. The metabolic effect of leptin in mice (inhibition of food intake, stimulation of energy consumption, both opposite to obesity, and amelioration of insulin resistance) only needs to be demonstrated in humans. However, many studies in humans indicate that leptin is a regulatory hormone that may be involved in various physiological and pathophysiological processes. The physiological factors that modulate the plasma level of leptin are sex, body fat, physical activity and modifications of calorie intake. The plasma level of leptin also shows a peripubertal increase and postmenopausal decrease. The hormones that elevate the concentration of leptin are insulin, glucocorticoids, oestradiol and growth hormone; whereas testosterone, somatostatin and insulin-like growth hormone I decrease it. End-stage renal disease is associated with a significant increase in plasma leptin. Proper understanding of the role and regulation of leptin in health and disease is a precondition for the rational use of a leptin-involving procedure in the management of metabolic disturbances in diabetic patients.

### 1.4.3. Thrifty genotype

The thrifty genotype hypothesis was proposed by Neel et al. as early as 1962. According to this hypothesis, type 2 diabetes, essential hypertension and obesity make the ‘syndrome of impaired genetic balance’. This hypothesis tries to explain why insulin resistance develops in some individuals, and why type 2 diabetes is more common in the individuals who have turned from the rural to the urban style of life. Neel et al. hypothesized that the thrifty genotype developed in individuals who had lived in very difficult conditions with uncertain food supply. Therefore, they had to increase their energy reserve excess to improve their chances for survival. Energy storage in the form of fat, especially intra-abdominal fat, is a more efficient way of energy storage for the body than glycogen storage in the muscle. This hypothesis has been supported by studies on obese mice (ob and db). Heterozygous animals (only homozygous animals will develop obesity and diabetes) of the same body weight and with the wild type had longer survival during starvation than insulin-sensitive mice with the

wild type. Sand rat is another example of such an insulin-resistant thrifty genotype, with a metabolism so adjusted as to preserve energy to ensure survival during the long periods of starvation in desert. Thrifty genotype could also be explained by asymmetric appetite regulation, which results in strict defence from body weight loss and weak defence from body weight gain. Obesity can provoke diabetes in genetically predisposed individuals. When a person with this energy-saving genotype is exposed, through relative affluence, to excess of food, typical of western society, the genotype becomes predominant causing glucose intolerance and type 2 diabetes.

### 1.5. Conclusion

The list of known and rarely occurring candidate genes associated with diabetes mellitus is very long and constantly increasing, pointing to the extreme genetic heterogeneity of the disease. Theoretically, there are as many potential candidate genes as hormones, receptors, enzymes, etc., included in the blood glucose regulation and related metabolic processes. However, all the presently recognized mutations can account for less than 1% of the aetiology of diabetes mellitus. To date, more than 250 candidate genes have been investigated, and results have shown a very high variability in gene association with diabetes mellitus: association for markers on chromosomes 2, 6, 10 and 11 was found in Mexican Americans; on chromosomes 1, 7 and 11 in Pima Indians; on chromosomes 1, 4, 7, 11 and 12 in Caucasians; and on chromosomes 12 and 20 in the Finns. Most of the published studies referred to the type 2 diabetes gene at locus 2q37, however, this association was not found in the Japanese, German and Italian studies.

Genetic studies have revealed not only different candidate genes for the development of diabetes mellitus in different populations, but also gene variability within the same population. Thus, the evidence for the involvement of several genes rather than a single gene in the genesis of diabetes mellitus appears to dim the prospects for possible use of gene therapy in the near future, with the exception of MODY, which is caused by one of the known genes and may therefore meet the expectations.

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