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(54) Title: HUMAN DIABETES SUSCEPTIBILITY TNFRSFIOB GENE

(57) Abstract: The present invention relates to a diagnostic method of determining whether a subject is at risk of developing type 2 diabetes, which method comprises detecting the presence of an alteration in the TNFRSFIOB gene locus in a biological sample of said subject.
The present invention relates to a method for determining a predisposition to diabetes in patients.

**BACKGROUND OF THE INVENTION**

According to the new etiologic classification of diabetes mellitus, four categories are differentiated: type 1 diabetes, type 2 diabetes, other specific types, and gestational diabetes mellitus (ADA, 2003). In the United States, Canada, and Europe, over 80% of cases of Diabetes are due to type 2 diabetes, 5 to 10% to type 1 diabetes, and the remainder to other specific causes.

In Type 1 diabetes, formerly known as insulin-dependent, the pancreas fails to produce the insulin which is essential for survival. This form develops most frequently in children and adolescents, but is being increasingly diagnosed later in life. Type 2 diabetes mellitus, formerly known as non-insulin dependent diabetes mellitus (NIDDM), or adult onset Diabetes, is the most common form of diabetes, accounting for approximately 90-95% of all diabetes cases. Type 2 diabetes is characterized by insulin resistance of peripheral tissues, especially muscle and liver, and primary or secondary insufficiency of insulin secretion from pancreatic beta-cells. Type 2 diabetes is defined by abnormally increased blood glucose levels and diagnosed if the fasting blood glucose level > 126 mg/dl (7.0 mmol/l) or blood glucose levels >200 mg/dl (11.0 mmol/l) 2 hours after an oral glucose uptake of 75g (oral glucose tolerance test, OGTT). Pre-diabetic states with already abnormal glucose values are defined as fasting hyperglycemia (FH) is superior to 6.1 mmol/l and <7.0 mmol/l or impaired glucose tolerance (IGT) are superior to 7.75 mmol/l and <11.0 mmol/l 2 hours after an OGTT.

Table 1: Classification of type 2 diabetes (WHO, 2006)

<table>
<thead>
<tr>
<th>Classification</th>
<th>Fasting blood glucose level (mmol/l)</th>
<th>2 hours after an OGTT (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normo glycemia</td>
<td>&lt; 7.0</td>
<td>&lt; 11.0</td>
</tr>
<tr>
<td>FH only</td>
<td>&gt; 6.1 to &lt; 7.0</td>
<td>&lt; 7.75</td>
</tr>
<tr>
<td>IGT only</td>
<td>&lt; 6.1</td>
<td>≥7.75 to &lt;11.0</td>
</tr>
<tr>
<td>FH and IGT</td>
<td>&gt; 6.1 to &lt; 7.0</td>
<td>≥7.75 to &lt;11.0</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>≥7.0</td>
<td>or ≥11.0</td>
</tr>
</tbody>
</table>
In 2000, there were approximately 171 million people, worldwide, with type 2 diabetes. The number of people with type 2 diabetes will expectedly more than double over the next 25 years, to reach a total of 366 million by 2030 (WHO/IDF, 2006). Most of this increase will occur as a result of a 150% rise in developing countries. In the US 7% of the general population are considered diabetic (over 15 million diabetics and an estimated 15 million people with impaired glucose tolerance).

Twin and adoption studies, marked ethnic differences in the incidence and prevalence of type 2 diabetes and the increase in incidence of type 2 diabetes in families suggest that heritable risk factors play a major role in the development of the disease. Known monogenic forms of diabetes are classified in two categories: genetic defects of the beta cell and genetic defects in insulin action (ADA, 2003). The diabetes forms associated with monogenetic defects in beta cell function are frequently characterized by onset of hyperglycemia at an early age (generally before age 25 years). They are referred to as maturity-onset diabetes of the Young (MODY) and are characterized by impaired insulin secretion with minimal or no defects in insulin action (Herman WH et al, 1994; Clement K et all, 1996; Byrne MM et all, 1996). They are inherited in an autosomal dominant pattern. Abnormalities at three genetic loci on different chromosomes have been identified to date. The most common form is associated with mutation on chromosome 12q in the locus of hepatic transcription factor referred to as hepatocyte nuclear factor (HNF)-4α (Vaxillaire M et all, 1995; Yamagata et all, 1996). A second form is associated with mutations in the locus of the glucokinase gene on chromosome 7q and result in a defective glucokinase molecule (Froguel P et all, 1992; viomnet N et all, 1992). Glucokinase converts glucose to glucose-6-phosphate, the metabolism of which, in turn, stimulates insulin secretion by the beta cell. Because of defects in the glucokinase gene, increased plasma levels of glucose are necessary to elicit normal levels of insulin secretion. A third form is associated with a mutation in the HnfMa gene on chromosome 20q (Bell GI et all, 1991; Yamagata K et all, 1996). HNF-4α is a transcription factor involved in the regulation of the expression of HNF-4α. Point mutations in mitochondrial DNA can cause diabetes mellitus primarily by impairing pancreatic beta cell function (Reardon W et all, 1992; VanDen Ouwenland JM et all, 1992; Kadowaki T et all, 1994). There are unusual causes of diabetes that result from genetically determined abnormalities of insulin action. The metabolic abnormalities
associated with mutation of the insulin receptor may range from hyperinsulinemia and modest hyperglycemia to severe diabetes (Kahn CR et al., 1976; Taylor SI, 1992).

Type 2 diabetes is a major risk factor for serious micro- and macro-vascular complications. The two major diabetic complications are cardiovascular disease, culminating in myocardial infarction. 50% of diabetics die of cardiovascular disease (primarily heart disease and stroke) and diabetic nephropathy. Diabetes is among the leading causes of kidney failure. 10-20% of people with diabetes die of kidney failure. Diabetic retinopathy is an important cause of blindness, and occurs as a result of long-term accumulated damage to the small blood vessels in the retina. After 15 years of diabetes, approximately 2% of people become blind, and about 10% develop severe visual impairment. Diabetic neuropathy is damage to the nerves as a result of diabetes, and affects up to 50% of all diabetics. Although many different problems can occur as a result of diabetic neuropathy, common symptoms are tingling, pain, numbness, or weakness in the feet and hands.

Combined with reduced blood flow, neuropathy in the feet increases the risk of foot ulcers and eventual limb amputation.

The two main contributors to the worldwide increase in prevalence of diabetes are population ageing and urbanization, especially in developing countries, with the consequent increase in the prevalence of obesity (WHO/IDF, 2006). Obesity is associated with insulin resistance and therefore a major risk factor for the development of type 2 diabetes. Obesity is defined as a condition of abnormal or excessive accumulation of adipose tissue, to the extent that health may be impaired. The body mass index (BMI; kg/m²) provides the most useful, albeit crude, population-level measure of obesity. Obesity has also been defined using the WHO classification of the different weight classes for adults.

<table>
<thead>
<tr>
<th>Classification</th>
<th>BMI (kg/m²)</th>
<th>Risk of co-morbidities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>&lt; 18.5</td>
<td>Low (but risks of other clinical problems increased)</td>
</tr>
<tr>
<td>Normal range</td>
<td>18.5 – 24.9</td>
<td>Average</td>
</tr>
<tr>
<td>Overweight</td>
<td>≥ 25</td>
<td></td>
</tr>
<tr>
<td>Pre-obese</td>
<td>25 – 29.9</td>
<td>Increased</td>
</tr>
<tr>
<td>Obese class I</td>
<td>30 – 34.9</td>
<td>Moderate</td>
</tr>
<tr>
<td>Obese class II</td>
<td>35 – 39.9</td>
<td>Severe</td>
</tr>
<tr>
<td>Obese class III</td>
<td>≥ 40</td>
<td>Very severe</td>
</tr>
</tbody>
</table>

Table 2: Classification of overweight in adults according to BMI (WHO, 2006)
More than 1 billion adults world-wide are considered overweight, with at least 300 million of them being clinically obese. Current obesity levels range from below 5% in China, Japan and certain African nations, to over 75% in urban Samoa. The prevalence of obesity is 10-25% in Western Europe and 20-27% in the Americas (WHO, 2006).

The rigorous control of balanced blood glucose levels is the foremost goal of all treatment in type 2 diabetes be it preventative or acute. Clinical intervention studies have shown that early intervention to decrease both obesity and/or pre-diabetic glucose levels through medication or lifestyle intervention, can reduce the risk to develop overt type 2 diabetes by up to 50% (Knowler WC et al, 2002). However, only 30% of obese individuals develop type 2 diabetes and the incentive for radical lifestyle intervention is often low as additional risk factors are lacking. Also, the diagnosis of type 2 diabetes through fasting blood glucose is insufficient to identify all individuals at risk for type 2 diabetes.

A further obstacle to rapidly achieve a balanced glucose homeostasis in diabetic patients is the multitude of therapeutic molecules with a wide range of response rates in the patients. Type 2 diabetes is treated either by oral application of anti-glycemic molecules or insulin injection. The oral antidiabetics either increase insulin secretion from the pancreatic beta-cells or that reduce the effects of the peripheral insulin resistance. Multiple rounds of differing treatments before an efficient treatment is found significantly decreases the compliance rates in diabetic patients.

Molecular and especially genetic tests hold the potential of identifying at risk individuals early, before onset of clinical symptoms and thereby the possibility for early intervention and prevention of the disease. They may also be useful in guiding treatment options thereby short-circuiting the need for long phases of sub-optimal treatment. Proof-of-principle has been shown for the treatment of individuals with maturity-onset diabetes of the young (MODY). Following molecular diagnosis many individuals with MODY3 or MODY2 can be put off insulin therapy and instead be treated with sulfonylureas (MODY 3) or adapted diet (MODY 2) respectively. Therefore, there is a need for a diagnostic test capable of evaluating the genetic risk factor associated with this disease. Such a test would be of great interest in order to adapt the lifestyle of people at risk and to prevent the onset of the disease.
SUMMARY OF THE INVENTION
The present invention now discloses the identification of a diabetes susceptibility gene. The invention thus provides a diagnostic method of determining whether a subject is at risk of developing type 2 diabetes, which method comprises detecting the presence of an alteration in the TNFRSFI0B gene locus in a biological sample of said subject. Specifically the invention pertains to single nucleotide polymorphisms in the TNFRSFI0B gene on chromosome 8 associated with type 2 diabetes.

LEGEND TO THE FIGURE
The Figure show High density mapping using Genomic Hybrid Identity Profiling (GenomeHIP). Graphical presentation of the linkage peak on chromosome 8p22-p2 1.2. The curve depict the linkage results for the GenomeHip procedure in the region. A total of 7 Bac clones on human chromosome 8 ranging from position p-ter-17.513.477 to 26.476.264-cen were tested for linkage using GenomeHip. Each point on the x-axis corresponds to a clone. Significant evidence for linkage was calculated for clone BACA12ZC07 (p-value 1.9E-10). The whole linkage region encompasses a region from 19.417.224 base pairs to 25.245.630 base pairs on human chromosome 8. The p-value less to 2x10^-5 corresponding to the significance level for significant linkage was used as a significance level for whole genome screens as proposed by Lander and Kruglyak (1995).

DETAILED DESCRIPTION OF THE INVENTION
The present invention discloses the identification of TNFRSFI0B as a diabetes susceptibility gene in individuals with type 2 diabetes. Various nucleic acid samples from diabetes families were submitted to a particular GenomeHIP process. This process led to the identification of particular identical-by-descent (IBD) fragments in said populations that are altered in diabetic subjects. By screening of the IBD fragments, the inventors identified the TNFRSFI0B gene as a candidate for type 2 diabetes. SNPs of the TNFRSFI0B gene were also identified, as being associated to type 2 diabetes.
DEFINITIONS
Type 2 diabetes is characterized by chronic hyperglycemia caused by pancreatic insulin secretion deficiency and/or insulin resistance of peripheral insulin sensitive tissues (e.g. muscle, liver). Long term hyperglycemia has been shown to lead to serious damage to various tissue including nerves tissue and blood vessels. Type 2 diabetes accounts for 90% all diabetes mellitus cases around the world (10% being type 1 diabetes characterized by the auto-immune destruction of the insulin producing pancreatic beta-cells). The invention described here pertains to a genetic risk factor for individuals to develop type 2 diabetes.

Within the context of this invention, the TNFRSFIOB gene locus designates all TNFRSFIOB sequences or products in a cell or organism, including TNFRSFIOB coding sequences, TNFRSFIOB non-coding sequences (e.g., introns), TNFRSFIOB regulatory sequences controlling transcription and/or translation (e.g., promoter, enhancer, terminator, etc.), as well as all corresponding expression products, such as TNFRSFIOB RNAs (e.g., mRNAs) and TNFRSFIOB polypeptides (e.g., a pre-protein and a mature protein). The TNFRSFIOB gene locus also comprise surrounding sequences of the TNFRSFIOB gene which include SNPs that are in linkage disequilibrium with SNPs located in the TNFRSFIOB gene.

As used in the present application, the term "TNFRSF 10B gene" designates the gene tumor necrosis factor receptor superfamily, member 10b, as well as variants or fragments thereof, including alleles thereof (e.g., germline mutations) which are related to susceptibility to type 2 diabetes. The TNFRSFIOB gene may also be referred to as CD262, DR5, KILLER, KILLER/DR5, TRAIL-R2, TRAILR2, TRICK2, TRICK2A, TRICK2B, TRICKB, ZTNFR9 or other designations like Fas-like protein precursor; TNF-related apoptosis-inducing ligand receptor 2; TRAIL receptor 2; apoptosis inducing protein TRICK2A/2B; apoptosis inducing receptor TRAIL-R2; cytotoxic TRAIL receptor-2; death domain containing receptor for TRAIL/Apo-2L; death receptor 5; p53-regulated DNA damage-inducible cell death receptor(killer); tumor necrosis factor receptor-like protein ZTNFR9. It is located on chromosome 8 at position 8p22-p2 1.

The cDNA sequence is shown as SEQ ID NO:1, and the protein as SEQ ID NO:2 (GenBank Source: AB054004).
The protein encoded by this gene is a member of the TNF-receptor superfamily, and contains an intracellular death domain. This receptor can be activated by tumor necrosis factor-related apoptosis inducing ligand (TNFSF10/TRAIL/APO-2L), and transduces apoptosis signal. Studies with FADD-deficient mice suggested that FADD, a death domain containing adaptor protein, is required for the apoptosis mediated by this protein.

The term "gene" shall be construed to include any type of coding nucleic acid, including genomic DNA (gDNA), complementary DNA (cDNA), synthetic or semi-synthetic DNA, as well as any form of corresponding RNA.

The TNFRSF1OB variants include, for instance, naturally-occurring variants due to allelic variations between individuals (e.g., polymorphisms), mutated alleles related to diabetes, alternative splicing forms, etc. The term variant also includes TNFRSF1OB gene sequences from other sources or organisms. Variants are preferably substantially homologous to SEQ ID No 1, i.e., exhibit a nucleotide sequence identity of at least about 65%, typically at least about 75%, preferably at least about 85%, more preferably at least about 95% with SEQ ID No 1. Variants of a TNFRSF1OB gene also include nucleic acid sequences, which hybridize to a sequence as defined above (or a complementary strand thereof) under stringent hybridization conditions. Typical stringent hybridization conditions include temperatures above 30°C, preferably above 35°C, more preferably in excess of 42°C, and/or salinity of less than about 500 mM, preferably less than 200 mM. Hybridization conditions may be adjusted by the skilled person by modifying the temperature, salinity and/or the concentration of other reagents such as SDS, SSC, etc.

A fragment of a TNFRSF1OB gene designates any portion of at least about 8 consecutive nucleotides of a sequence as disclosed above, preferably at least about 15, more preferably at least about 20 nucleotides, further preferably of at least 30 nucleotides. Fragments include all possible nucleotide lengths between 8 and 100 nucleotides, preferably between 15 and 100, more preferably between 20 and 100.
A TNFRSF1OB polypeptide designates any protein or polypeptide encoded by a TNFRSF1OB gene as disclosed above. The term "polypeptide" refers to any molecule comprising a stretch of amino acids. This term includes molecules of various lengths, such as peptides and proteins. The polypeptide may be modified, such as by glycosylations and/or acetylations and/or chemical reaction or coupling, and may contain one or several non-natural or synthetic amino acids. A specific example of a TNFRSF1OB polypeptide comprises all or part of SEQ ID No: 2.

DIAGNOSIS

The invention now provides diagnosis methods based on a monitoring of the TNFRSF1OB gene locus in a subject. Within the context of the present invention, the term 'diagnosis" includes the detection, monitoring, dosing, comparison, etc., at various stages, including early, pre-symptomatic stages, and late stages, in adults or children. Diagnosis typically includes the prognosis, the assessment of a predisposition or risk of development, the characterization of a subject to define most appropriate treatment (pharmacogenetics), etc.

The present invention provides diagnostic methods to determine whether a subject, is at risk of developing type 2 diabetes resulting from a mutation or a polymorphism in the TNFRSF1OB gene locus.

It is therefore provided a method of detecting the presence of or predisposition to type 2 diabetes in a subject, the method comprising detecting in a biological sample from the subject the presence of an alteration in the TNFRSF1OB gene locus in said sample. The presence of said alteration is indicative of the presence or predisposition to type 2 diabetes. Optionally, said method comprises a preliminary step of providing a sample from a subject. Preferably, the presence of an alteration in the TNFRSF1OB gene locus in said sample is detected through the genotyping of a sample.

In a preferred embodiment, said alteration is one or several SNP(s) or a haplotype of SNPs associated with type 2 diabetes. More preferably, said SNP associated with type 2 diabetes is as shown in Table 3A.
In a preferred embodiment, said SNP is selected from the group consisting of SNP271, SNP272, SNP278, SNP280, SNP281, and SNP282.

Other SNP(s), as listed in Table 3B, may be informative too.
Table 3A: SNPs on TNFRSF1OB gene associated with type 2 diabetes (Int:Intron):

<table>
<thead>
<tr>
<th>SNP identity</th>
<th>dbSNP reference</th>
<th>Allele1</th>
<th>Allele2</th>
<th>Frequency Allele1 from CEU HapMap</th>
<th>Frequency Allele2 from CEU HapMap</th>
<th>Nucleotide position in genomic sequence of chromosome 8 based on NCBI Build 35</th>
<th>Position in locus</th>
<th>SEQ ID NO:</th>
</tr>
</thead>
<tbody>
<tr>
<td>271</td>
<td>rs1001793</td>
<td>A=1</td>
<td>G=2</td>
<td>0.233</td>
<td>0.767</td>
<td>22956894</td>
<td>Intron1</td>
<td>3</td>
</tr>
<tr>
<td>272</td>
<td>rs12677679</td>
<td>A=1</td>
<td>G=2</td>
<td>0.808</td>
<td>0.192</td>
<td>22967018</td>
<td>Intron 1</td>
<td>4</td>
</tr>
<tr>
<td>273</td>
<td>rs10866819</td>
<td>A=1</td>
<td>G=2</td>
<td>0.623</td>
<td>0.377</td>
<td>22973638</td>
<td>Intron 1</td>
<td>5</td>
</tr>
<tr>
<td>278</td>
<td>rs7830593</td>
<td>A=1</td>
<td>G=2</td>
<td>0.2</td>
<td>0.8</td>
<td>23000640</td>
<td>5’</td>
<td>6</td>
</tr>
<tr>
<td>280</td>
<td>rs4518666</td>
<td>C=1</td>
<td>T=2</td>
<td>0.351</td>
<td>0.649</td>
<td>23010150</td>
<td>5’</td>
<td>7</td>
</tr>
<tr>
<td>281</td>
<td>rs4871846</td>
<td>C=1</td>
<td>G=2</td>
<td>0.608</td>
<td>0.392</td>
<td>23011252</td>
<td>5’</td>
<td>8</td>
</tr>
<tr>
<td>282</td>
<td>rs12545733</td>
<td>C=1</td>
<td>T=2</td>
<td>0.833</td>
<td>0.167</td>
<td>23012948</td>
<td>5’</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 3B: Other SNPs on TNFRSF1OB gene (Int:Intron):

<table>
<thead>
<tr>
<th>SNP identity</th>
<th>dbSNP reference</th>
<th>Allele1</th>
<th>Allele2</th>
<th>Frequency Allele1 from CEU HapMap</th>
<th>Frequency Allele2 from CEU HapMap</th>
<th>Nucleotide position in genomic sequence of chromosome 8 based on NCBI Build 35</th>
<th>Position in locus</th>
<th>SEQ ID NO:</th>
</tr>
</thead>
<tbody>
<tr>
<td>266</td>
<td>rs1047275</td>
<td>C=1</td>
<td>G=2</td>
<td>0.475</td>
<td>0.525</td>
<td>22936107</td>
<td>3’</td>
<td>10</td>
</tr>
<tr>
<td>267</td>
<td>rs883429</td>
<td>C=1</td>
<td>T=2</td>
<td>0.658</td>
<td>0.342</td>
<td>22942763</td>
<td>Intron 4</td>
<td>11</td>
</tr>
<tr>
<td>269</td>
<td>rs1178599</td>
<td>C=1</td>
<td>T=2</td>
<td>0.45</td>
<td>0.55</td>
<td>22948219</td>
<td>Intron 2</td>
<td>12</td>
</tr>
<tr>
<td>270</td>
<td>rs7834266</td>
<td>C=1</td>
<td>T=2</td>
<td>0.617</td>
<td>0.383</td>
<td>22954349</td>
<td>Intron 2</td>
<td>13</td>
</tr>
<tr>
<td>274</td>
<td>rs4424253</td>
<td>C=1</td>
<td>T=2</td>
<td>0.833</td>
<td>0.167</td>
<td>22975267</td>
<td>Intron 1</td>
<td>14</td>
</tr>
<tr>
<td>275</td>
<td>rs11135693</td>
<td>A=1</td>
<td>C=2</td>
<td>0.408</td>
<td>0.592</td>
<td>22981099</td>
<td>Intron 1</td>
<td>15</td>
</tr>
<tr>
<td>276</td>
<td>rs4872049</td>
<td>C=1</td>
<td>T=2</td>
<td>0.5</td>
<td>0.5</td>
<td>22987724</td>
<td>5’</td>
<td>16</td>
</tr>
<tr>
<td>279</td>
<td>rs12678837</td>
<td>A=1</td>
<td>G=2</td>
<td>0.1</td>
<td>0.9</td>
<td>23006334</td>
<td>5’</td>
<td>17</td>
</tr>
</tbody>
</table>
Preferably the SNP is allele G of SNP271 and allele C of SNP280.

More preferably, said haplotype comprises or consists of several SNPs selected from the group consisting of SNP271, SNP272, SNP280, SNP282, more particularly the following haplotype:

2 - 1 - 1 - 1  (i.e. SNP271 is G, SNP272 is A, SNP280 is C and SNP282 is C).

The invention further provides a method for preventing type 2 diabetes in a subject, comprising detecting the presence of an alteration in the TNFRSF1OB gene locus in a sample from the subject, the presence of said alteration being indicative of the predisposition to type 2 diabetes, and administering a prophylactic treatment against type 2 diabetes.

The alteration may be determined at the level of the TNFRSF1OB gDNA, RNA or polypeptide. Optionally, the detection is performed by sequencing all or part of the TNFRSF1OB gene or by selective hybridization or amplification of all or part of the TNFRSF1OB gene. More preferably a TNFRSF1OB gene specific amplification is carried out before the alteration identification step.

An alteration in the TNFRSF1OB gene locus may be any form of mutation(s), deletion(s), rearrangement(s) and/or insertions in the coding and/or non-coding region of the locus, alone or in various combination(s). Mutations more specifically include point mutations. Deletions may encompass any region of two or more residues in a coding or non-coding portion of the gene locus, such as from two residues up to the entire gene or locus. Typical deletions affect smaller regions, such as domains (introns) or repeated sequences or fragments of less than about 50 consecutive base pairs, although larger deletions may occur as well. Insertions may encompass the addition of one or several residues in a coding or non-coding portion of the gene locus. Insertions may typically comprise an addition of between 1 and 50 base pairs in the gene locus. Rearrangement includes inversion of sequences. The TNFRSF1OB gene locus alteration may result in the creation of stop codons, frameshift mutations, amino acid substitutions, particular RNA splicing or
processing, product instability, truncated polypeptide production, etc. The alteration may result in the production of a TNFRSF1OB polypeptide with altered function, stability, targeting or structure. The alteration may also cause a reduction in protein expression or, alternatively, an increase in said production.

In a particular embodiment of the method according to the present invention, the alteration in the TNFRSF1OB gene locus is selected from a point mutation, a deletion and an insertion in the TNFRSF1OB gene or corresponding expression product, more preferably a point mutation and a deletion.

In any method according to the present invention, one or several SNP in the TNFRSF1OB gene and certain haplotypes comprising SNP in the TNFRSF1OB gene can be used in combination with other SNP or haplotype associated with type 2 diabetes and located in other gene(s).

In another variant, the method comprises detecting the presence of an altered TNFRSF1OB RNA expression. Altered RNA expression includes the presence of an altered RNA sequence, the presence of an altered RNA splicing or processing, the presence of an altered quantity of RNA, etc. These may be detected by various techniques known in the art, including by sequencing all or part of the TNFRSF1OB RNA or by selective hybridization or selective amplification of all or part of said RNA, for instance.

In a further variant, the method comprises detecting the presence of an altered TNFRSF1OB polypeptide expression. Altered TNFRSF1OB polypeptide expression includes the presence of an altered polypeptide sequence, the presence of an altered quantity of TNFRSF1OB polypeptide, the presence of an altered tissue distribution, etc. These may be detected by various techniques known in the art, including by sequencing and/or binding to specific ligands (such as antibodies), for instance.

As indicated above, various techniques known in the art may be used to detect or quantify altered TNFRSF1OB gene or RNA expression or sequence, including sequencing,
hybridization, amplification and/or binding to specific ligands (such as antibodies). Other suitable methods include allele-specific oligonucleotide (ASO), allele-specific amplification, Southern blot (for DNAs), Northern blot (for RNAs), single-stranded conformation analysis (SSCA), PFGE, fluorescent in situ hybridization (FISH), gel migration, clamped denaturing gel electrophoresis, heteroduplex analysis, RNase protection, chemical mismatch cleavage, ELISA, radio-immunoassays (RIA) and immuno-enzymatic assays (IEMA).

Some of these approaches (e.g., SSCA and CGGE) are based on a change in electrophoretic mobility of the nucleic acids, as a result of the presence of an altered sequence. According to these techniques, the altered sequence is visualized by a shift in mobility on gels. The fragments may then be sequenced to confirm the alteration.

Some others are based on specific hybridization between nucleic acids from the subject and a probe specific for wild type or altered TNFRSFIOB gene or RNA. The probe may be in suspension or immobilized on a substrate. The probe is typically labeled to facilitate detection of hybrids.

Some of these approaches are particularly suited for assessing a polypeptide sequence or expression level, such as Northern blot, ELISA and RIA. These latter require the use of a ligand specific for the polypeptide, more preferably of a specific antibody.

In a particular, preferred, embodiment, the method comprises detecting the presence of an altered TNFRSFIOB gene expression profile in a sample from the subject. As indicated above, this can be accomplished more preferably by sequencing, selective hybridization and/or selective amplification of nucleic acids present in said sample.

**Sequencing**

Sequencing can be carried out using techniques well known in the art, using automatic sequencers. The sequencing may be performed on the complete TNFRSFIOB gene or, more
preferably, on specific domains thereof, typically those known or suspected to carry deleterious mutations or other alterations.

Amplification

Amplification is based on the formation of specific hybrids between complementary nucleic acid sequences that serve to initiate nucleic acid reproduction.

Amplification may be performed according to various techniques known in the art, such as by polymerase chain reaction (PCR), ligase chain reaction (LCR), strand displacement amplification (SDA) and nucleic acid sequence based amplification (NASBA). These techniques can be performed using commercially available reagents and protocols. Preferred techniques use allele-specific PCR or PCR-SSCP. Amplification usually requires the use of specific nucleic acid primers, to initiate the reaction.

Nucleic acid primers useful for amplifying sequences from the TNFRSF1OB gene or locus are able to specifically hybridize with a portion of the TNFRSF1OB gene locus that flank a target region of said locus, said target region being altered in certain subjects having type 2 diabetes. Examples of such target regions are provided in Table 3A or Table 3B.

Primers that can be used to amplify TNFRSF1OB target region comprising SNPs as identified in Table 3A or Table 3B may be designed based on the sequence of SEQ ID No 1 or on the genomic sequence of TNFRSF1OB. In a particular embodiment, primers may be designed based on the sequence of SEQ ID Nos 23-53.

Typical primers of this invention are single-stranded nucleic acid molecules of about 5 to 60 nucleotides in length, more preferably of about 8 to about 25 nucleotides in length. The sequence can be derived directly from the sequence of the TNFRSF1OB gene locus. Perfect complementarity is preferred, to ensure high specificity. However, certain mismatch may be tolerated.
The invention also concerns the use of a nucleic acid primer or a pair of nucleic acid primers as described above in a method of detecting the presence of or predisposition to type 2 diabetes in a subject.

Selective hybridization

Hybridization detection methods are based on the formation of specific hybrids between complementary nucleic acid sequences that serve to detect nucleic acid sequence alteration(s).

A particular detection technique involves the use of a nucleic acid probe specific for wild type or altered TNFRSF1OB gene or RNA, followed by the detection of the presence of a hybrid. The probe may be in suspension or immobilized on a substrate or support (as in nucleic acid array or chips technologies). The probe is typically labeled to facilitate detection of hybrids.

In this regard, a particular embodiment of this invention comprises contacting the sample from the subject with a nucleic acid probe specific for an altered TNFRSF1OB gene locus, and assessing the formation of an hybrid. In a particular, preferred embodiment, the method comprises contacting simultaneously the sample with a set of probes that are specific, respectively, for wild type TNFRSF1OB gene locus and for various altered forms thereof.

In this embodiment, it is possible to detect directly the presence of various forms of alterations in the TNFRSF1OB gene locus in the sample. Also, various samples from various subjects may be treated in parallel.

Within the context of this invention, a probe refers to a polynucleotide sequence which is complementary to and capable of specific hybridization with a (target portion of a) TNFRSF1OB gene or RNA, and which is suitable for detecting polynucleotide polymorphisms associated with TNFRSF1OB alleles which predispose to or are associated with obesity or an associated disorder. Probes are preferably perfectly complementary to the TNFRSF1OB gene, RNA, or target portion thereof. Probes typically comprise single-stranded nucleic acids of between 8 to 1000 nucleotides in length, for instance of between
10 and 800, more preferably of between 15 and 700, typically of between 20 and 500. It should be understood that longer probes may be used as well. A preferred probe of this invention is a single stranded nucleic acid molecule of between 8 to 500 nucleotides in length, which can specifically hybridise to a region of a TNFRSFlOB gene or RNA that carries an alteration.

A specific embodiment of this invention is a nucleic acid probe specific for an altered (e.g., a mutated) TNFRSFlOB gene or RNA, i.e., a nucleic acid probe that specifically hybridises to said altered TNFRSFlOB gene or RNA and essentially does not hybridise to a TNFRSFlOB gene or RNA lacking said alteration. Specificity indicates that hybridization to the target sequence generates a specific signal which can be distinguished from the signal generated through non-specific hybridization. Perfectly complementary sequences are preferred to design probes according to this invention. It should be understood, however, that a certain degree of mismatch may be tolerated, as long as the specific signal may be distinguished from non-specific hybridization.

Particular examples of such probes are nucleic acid sequences complementary to a target portion of the genomic region including the TNFRSFlOB gene or RNA carrying a point mutation as listed in Table 3A or Table 3B above. More particularly, the probes can comprise a sequence selected from the group consisting of SEQ ID Nos 23-53 or a fragment thereof comprising the SNP or a complementary sequence thereof.

The sequence of the probes can be derived from the sequences of the TNFRSFlOB gene and RNA as provided in the present application. Nucleotide substitutions may be performed, as well as chemical modifications of the probe. Such chemical modifications may be accomplished to increase the stability of hybrids (e.g., intercalating groups) or to label the probe. Typical examples of labels include, without limitation, radioactivity, fluorescence, luminescence, enzymatic labeling, etc.

The invention also concerns the use of a nucleic acid probe as described above in a method of detecting the presence of or predisposition to type 2 diabetes in a subject or in a method
of assessing the response of a subject to a treatment of type 2 diabetes or an associated disorder.

**Specific Ligand Binding**

As indicated above, alteration in the TNFRSF1OB gene locus may also be detected by screening for alteration(s) in TNFRSF1OB polypeptide sequence or expression levels. In this regard, a specific embodiment of this invention comprises contacting the sample with a ligand specific for a TNFRSF1OB polypeptide and determining the formation of a complex.

Different types of ligands may be used, such as specific antibodies. In a specific embodiment, the sample is contacted with an antibody specific for a TNFRSF1OB polypeptide and the formation of an immune complex is determined. Various methods for detecting an immune complex can be used, such as ELISA, radioimmunoassays (RIA) and immuno-enzymatic assays (IEMA).

Within the context of this invention, an antibody designates a polyclonal antibody, a monoclonal antibody, as well as fragments or derivatives thereof having substantially the same antigen specificity. Fragments include Fab, Fab'2, CDR regions, etc. Derivatives include single-chain antibodies, humanized antibodies, poly-functional antibodies, etc.

An antibody specific for a TNFRSF1OB polypeptide designates an antibody that selectively binds a TNFRSF1OB polypeptide, namely, an antibody raised against a TNFRSF1OB polypeptide or an epitope-containing fragment thereof. Although non-specific binding towards other antigens may occur, binding to the target TNFRSF1OB polypeptide occurs with a higher affinity and can be reliably discriminated from non-specific binding.

In a specific embodiment, the method comprises contacting a sample from the subject with (a support coated with) an antibody specific for an altered form of a TNFRSF1OB polypeptide, and determining the presence of an immune complex. In a particular embodiment, the sample may be contacted simultaneously, or in parallel, or sequentially,
with various (supports coated with) antibodies specific for different forms of a TNFRSF1OB polypeptide, such as a wild type and various altered forms thereof.

The invention also concerns the use of a ligand, preferably an antibody, a fragment or a derivative thereof as described above, in a method of detecting the presence of or predisposition to type 2 diabetes in a subject.

In order to carry out the methods of the invention, one can employ diagnostic kits comprising products and reagents for detecting in a sample from a subject the presence of an alteration in the TNFRSF1OB gene or polypeptide, in the TNFRSF1OB gene or polypeptide expression, and/or in TNFRSF1OB activity. Said diagnostic kit comprises any primer, any pair of primers, any nucleic acid probe and/or any ligand, preferably antibody, described in the present invention. Said diagnostic kit can further comprise reagents and/or protocols for performing a hybridization, amplification or antigen-antibody immune reaction.

The diagnosis methods can be performed in vitro, ex vivo or in vivo, preferably in vitro or ex vivo. They use a sample from the subject, to assess the status of the TNFRSF1OB gene locus. The sample may be any biological sample derived from a subject, which contains nucleic acids or polypeptides. Examples of such samples include fluids, tissues, cell samples, organs, biopsies, etc. Most preferred samples are blood, plasma, saliva, urine, seminal fluid, etc. The sample may be collected according to conventional techniques and used directly for diagnosis or stored. The sample may be treated prior to performing the method, in order to render or improve availability of nucleic acids or polypeptides for testing. Treatments include, for instance, lysis (e.g., mechanical, physical, chemical, etc.), centrifugation, etc. Also, the nucleic acids and/or polypeptides may be pre-purified or enriched by conventional techniques, and/or reduced in complexity. Nucleic acids and polypeptides may also be treated with enzymes or other chemical or physical treatments to produce fragments thereof. Considering the high sensitivity of the claimed methods, very few amounts of sample are sufficient to perform the assay.
As indicated, the sample is preferably contacted with reagents such as probes, primers or ligands in order to assess the presence of an altered TNFRSFI0B gene locus. Contacting may be performed in any suitable device, such as a plate, tube, well, glass, etc. In specific embodiments, the contacting is performed on a substrate coated with the reagent, such as a nucleic acid array or a specific ligand array. The substrate may be a solid or semi-solid substrate such as any support comprising glass, plastic, nylon, paper, metal, polymers and the like. The substrate may be of various forms and sizes, such as a slide, a membrane, a bead, a column, a gel, etc. The contacting may be made under any condition suitable for a complex to be formed between the reagent and the nucleic acids or polypeptides of the sample.

The finding of an altered TNFRSFI0B polypeptide, RNA or DNA in the sample is indicative of the presence of an altered TNFRSFI0B gene locus in the subject, which can be correlated to the presence, predisposition or stage of progression of type 2 diabetes. For example, an individual having a germ line TNFRSFI0B mutation has an increased risk of developing type 2 diabetes. The determination of the presence of an altered TNFRSFI0B gene locus in a subject also allows the design of appropriate therapeutic intervention, which is more effective and customized.

**Linkage Disequilibrium**

Once a first SNP has been identified in a genomic region of interest, more particularly in TNFRSFI0B gene locus, the practitioner of ordinary skill in the art can easily identify additional SNPs in linkage disequilibrium with this first SNP. Indeed, any SNP in linkage disequilibrium with a first SNP associated with type 2 diabetes will be associated with this trait. Therefore, once the association has been demonstrated between a given SNP and type 2 diabetes, the discovery of additional SNPs associated with this trait can be of great interest in order to increase the density of SNPs in this particular region.

Identification of additional SNPs in linkage disequilibrium with a given SNP involves: (a) amplifying a fragment from the genomic region comprising or surrounding a first SNP from a plurality of individuals; (b) identifying of second SNPs in the genomic region
harboring or surrounding said first SNP; (c) conducting a linkage disequilibrium analysis between said first SNP and second SNPs; and (d) selecting said second SNPs as being in linkage disequilibrium with said first marker. Subcombinations comprising steps (b) and (c) are also contemplated.

Methods to identify SNPs and to conduct linkage disequilibrium analysis can be carried out by the skilled person without undue experimentation by using well-known methods.

These SNPs in linkage disequilibrium can also be used in the methods according to the present invention, and more particularly in the diagnostic methods according to the present invention.

For example, a linkage locus of Crohn's disease has been mapped to a large region spanning 18cM on chromosome 5q31 (Rioux et al., 2000 and 2001). Using dense maps of microsatellite markers and SNPs across the entire region, strong evidence of linkage disequilibrium (LD) was found. Having found evidence of LD, the authors developed an ultra-high-density SNP map and studied a denser collection of markers selected from this map. Multilocus analyses defined a single common risk haplotype characterised by multiple SNPs that were each independently associated using TDT. These SNPs were unique to the risk haplotype and essentially identical in their information content by virtue of being in nearly complete LD with one another. The equivalent properties of these SNPs make it impossible to identify the causal mutation within this region on the basis of genetic evidence alone.

**Causal Mutation**

Mutations in the TNFRSF1OB gene which are responsible for type 2 diabetes may be identified by comparing the sequences of the TNFRSF1OB gene from patients presenting type 2 diabetes and control individuals. Based on the identified association of SNPs of TNFRSF1OB and type 2 diabetes, the identified locus can be scanned for mutations. In a preferred embodiment, functional regions such as exons and splice sites, promoters and other regulatory regions of the TNFRSF1OB gene are scanned for mutations. Preferably,
patients presenting type 2 diabetes carry the mutation shown to be associated with type 2 diabetes and controls individuals do not carry the mutation or allele associated with type 2 diabetes or an associated disorder. It might also be possible that patients presenting type 2 diabetes carry the mutation shown to be associated with type 2 diabetes with a higher frequency than controls individuals.

The method used to detect such mutations generally comprises the following steps: amplification of a region of the TNFRSF1OB gene comprising a SNP or a group of SNPs associated with type 2 diabetes from DNA samples of the TNFRSF1OB gene from patients presenting type 2 diabetes and control individuals; sequencing of the amplified region; comparison of DNA sequences of the TNFRSF1OB gene from patients presenting type 2 diabetes and control individuals; determination of mutations specific to patients presenting type 2 diabetes.

Therefore, identification of a causal mutation in the TNFRSF1OB gene can be carried out by the skilled person without undue experimentation by using well-known methods.

For example, the causal mutations have been identified in the following examples by using routine methods.

Hugot et al. (2001) applied a positional cloning strategy to identify gene variants with susceptibility to Crohn's disease in a region of chromosome 16 previously found to be linked to susceptibility to Crohn's disease. To refine the location of the potential susceptibility locus 26 microsatellite markers were genotyped and tested for association to Crohn's disease using the transmission disequilibrium test. A borderline significant association was found between one allele of the microsatellite marker D16S136. Eleven additional SNPs were selected from surrounding regions and several SNPs showed significant association. SNP5-8 from this region were found to be present in a single exon of the NOD2/CARD15 gene and shown to be non-synonymous variants. This prompted the authors to sequence the complete coding sequence of this gene in 50 CD patients. Two additional non-synonymous mutations (SNP12 and SNP13) were found. SNP13 was most significant associated
(p=6x10^-6) using the pedigree transmission disequilibrium test. In another independent study, the same variant was found also by sequencing the coding region of this gene from 12 affected individuals compared to 4 controls (Ogura et al., 2001). The rare allele of SNP13 corresponded to a 1-bp insertion predicted to truncate the NOD2/CARD15 protein. This allele was also present in normal healthy individuals, albeit with significantly lower frequency as compared to the controls.

Similarly, Lesage et al. (2002) performed a mutational analyses of CARD15 in 453 patients with CD, including 166 sporadic and 287 familial cases, 159 patients with ulcerative colitis (UC), and 103 healthy control subjects by systematic sequencing of the coding region. Of 67 sequence variations identified, 9 had an allele frequency >5% in patients with CD. Six of them were considered to be polymorphisms, and three (SNP12-R702W, SNP8-G908R, and SNP13-1007fs) were confirmed to be independently associated with susceptibility to CD. Also considered as potential disease-causing mutations (DCMs) were 27 rare additional mutations. The three main variants (R702W, G908R, and 1007fs) represented 32%, 18%, and 31%, respectively, of the total CD mutations, whereas the total of the 27 rare mutations represented 19% of DCMs. Altogether, 93% of the mutations were located in the distal third of the gene. No mutations were found to be associated with UC. In contrast, 50% of patients with CD carried at least one DCM, including 17% who had a double mutation.

The present invention demonstrates the correlation between type 2 diabetes and the TNFRSFIOB gene locus. The invention thus provides a novel target of therapeutic intervention. Various approaches can be contemplated to restore or modulate the TNFRSFIOB activity or function in a subject, particularly those carrying an altered TNFRSFIOB gene locus. Supplying wild-type function to such subjects is expected to suppress phenotypic expression of type 2 diabetes in a pathological cell or organism. The supply of such function can be accomplished through gene or protein therapy, or by administering compounds that modulate or mimic TNFRSFIOB polypeptide activity (e.g., agonists as identified in the above screening assays).
Other molecules with TNFRSFIOB activity (e.g., peptides, drugs, TNFRSFIOB agonists, or organic compounds) may also be used to restore functional TNFRSFIOB activity in a subject or to suppress the deleterious phenotype in a cell. Restoration of functional TNFRSFIOB gene function in a cell may be used to prevent the development of type 2 diabetes or to reduce progression of said diseases. Such a treatment may suppress the type 2 diabetes-associated phenotype of a cell, particularly those cells carrying a deleterious allele.

Further aspects and advantages of the present invention will be disclosed in the following experimental section, which should be regarded as illustrative and not limiting the scope of the present application.

**EXAMPLES**

1. GenomeHIP platform to identify the chromosome 8 susceptibility gene

The GenomeHIP platform was applied to allow rapid identification of a type 2 diabetes susceptibility gene.

Briefly, the technology consists of forming pairs from the DNA of related individuals. Each DNA is marked with a specific label allowing its identification. Hybrids are then formed between the two DNAs. A particular process (WO00/53802) is then applied that selects all fragments identical-by-descent (IBD) from the two DNAs in a multi-step procedure. The remaining IBD enriched DNA is then scored against a BAC clone derived DNA microarray that allows the positioning of the IBD fraction on a chromosome.

The application of this process over many different families results in a matrix of IBD fractions for each pair from each family. Statistical analyses then calculate the minimal IBD regions that are shared between all families tested. Significant results (p-values) are evidence for linkage of the positive region with the trait of interest (here type 2 diabetes). The linked interval can be delimited by the two most distant clones showing significant p-values.
In the present study, 119 diabetes (type 2 diabetes) relative pairs, were submitted to the GenomeHIP process. The resulting IBD enriched DNA fractions were then labelled with Cy5 fluorescent dyes and hybridised against a DNA array consisting of 2263 BAC clones covering the whole human genome with an average spacing of 1.2 Mega base pairs. Non-selected DNA labelled with Cy3 was used to normalize the signal values and compute ratios for each clone. Clustering of the ratio results was then performed to determine the IBD status for each clone and pair.

By applying this procedure, several BAC clones spanning approximately 4.5 Mega bases in the region on chromosome 8 were identified, that showed significant evidence for linkage to type 2 diabetes (p=1.90E-10).

2. Identification of a type 2 diabetes susceptibility gene on chromosome 8

By screening the aforementioned 5.8 Megabases in the linked chromosomal region, the inventors identified the TNFRSF1OB gene as a candidate for type 2 diabetes. This gene is indeed present in the critical interval, with evidence for linkage delimited by the clones outlined above.

Table 4: Linkage results for chromosome 8 in the TNFRSF1OB locus: Indicated is the region correspondent to BAC clones with evidence for linkage. The start and stop positions of the clones correspond to their genomic location based on NCBI Build 35 sequence respective to the start of the chromosome (p-ter).

<table>
<thead>
<tr>
<th>Human chrom.</th>
<th>Clone Name (Origin name)</th>
<th>Start</th>
<th>Stop</th>
<th>% of informative pairs</th>
<th>IBD sharing (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>BACA12ZD05 (RP11-499D5)</td>
<td>17,513,477</td>
<td>17,685,793</td>
<td>60%</td>
<td>0.83</td>
<td>7.1 * 10^-2</td>
</tr>
<tr>
<td>8</td>
<td>BACA11ZA04 (RP11-51C1)</td>
<td>19,416,907</td>
<td>19,417,225</td>
<td>76%</td>
<td>0.86</td>
<td>1.1 * 10^-2</td>
</tr>
<tr>
<td>8</td>
<td>BACA12ZD06</td>
<td>20,134,018</td>
<td>20,300,107</td>
<td>63%</td>
<td>7.6 * 10^-6</td>
<td></td>
</tr>
</tbody>
</table>
Taken together, the linkage results provided in the present application, identifying the human TNFRSF1OB gene in the critical interval of genetic alterations linked to type 2 diabetes on chromosome 8.

3. **Association study**

*Single SNP and haplotype analysis :*

Differences in allele distributions between 1034 cases and 1034 controls were screened for all SNPs. Association analyses have been conducted using COCAPHASE v2.404 software from the UNPHASED suite of programs.

The method is based on likelihood ratio tests in a logistic model:

$$\log\left(\frac{n}{1-p}\right) = mu + \sum \beta_i X_i,$$

where p is the probability of a chromosome being a "case" rather than a "control", $X_i$ are variables which represent the allele or haplotypes in some way depending upon the particular test, and mu and $\beta_i$ are coefficients to be estimated. Reference for this application of log-linear models is Cordell & Clayton, AJHG (2002)

In cases of uncertain haplotype, the method for case-control sample is a standard unconditional logistic regression identical to the model-free method T5 of EHPLUS (Zhao et al Hum Hered (2000) and the log-linear modelling of Mander. The $\beta_i$ are log odds ratios for the haplotypes. The EM algorithm is used to obtain maximum likelihood frequency estimates.
SNP Genotype analysis:

Differences in genotype distributions between cases and controls were screened for all SNPs. For each SNPs, three genotype is possible genotype \( RR \), genotype \( Rn \) and genotype \( nn \) where R represented the associate allele of the SNP with TYPE 2 DIABETES. Dominant transmission model for associated risk allele (R) vs the non-risk allele (n) were tested by counting \( nRa \) and \( RR \) genotype together. The statistic test was carried out using the standard Chi-square independence test with 1 df (genotype distribution, 2x2 table). Recessive transmission model for associated allele (R) were tested by counting the non-risk \( nn \) and \( nR \) genotypes together. The statistic test was carried out using the standard Chi-square independence test with 1 df (genotype distribution, 2x2 table). Additive transmission model for associated allele (a) were tested using the standard Chi-square independence test with 2 df (genotype distribution, 2x3 table).

### 3.1 - Association with single SNPs, allele frequencies statistics test:

<table>
<thead>
<tr>
<th>SNP identity</th>
<th>dbSNP reference</th>
<th>Allele</th>
<th>Cases</th>
<th>Frequency in Cases</th>
<th>Controls</th>
<th>Frequency in Controls</th>
<th>Risk Allele</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>271</td>
<td>Rs1001793</td>
<td>1</td>
<td>558</td>
<td>0.27</td>
<td>633</td>
<td>0.31</td>
<td>G</td>
<td>0.007814</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>1498</td>
<td>0.73</td>
<td>1415</td>
<td>0.69</td>
<td></td>
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</tr>
<tr>
<td>272</td>
<td>Rs12677679</td>
<td>1</td>
<td>1606</td>
<td>0.78</td>
<td>1533</td>
<td>0.75</td>
<td>A</td>
<td>0.013800</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>450</td>
<td>0.22</td>
<td>515</td>
<td>0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>278</td>
<td>Rs7830593</td>
<td>1</td>
<td>491</td>
<td>0.24</td>
<td>427</td>
<td>0.21</td>
<td>A</td>
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<tr>
<td></td>
<td></td>
<td>2</td>
<td>1559</td>
<td>0.76</td>
<td>1619</td>
<td>0.79</td>
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<tr>
<td>280</td>
<td>Rs4518666</td>
<td>1</td>
<td>829</td>
<td>0.41</td>
<td>726</td>
<td>0.36</td>
<td>C</td>
<td>0.001170</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>1217</td>
<td>0.59</td>
<td>1314</td>
<td>0.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>281</td>
<td>Rs4871846</td>
<td>1</td>
<td>1166</td>
<td>0.58</td>
<td>1241</td>
<td>0.61</td>
<td>G</td>
<td>0.026700</td>
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<tr>
<td></td>
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<td>2</td>
<td>846</td>
<td>0.42</td>
<td>781</td>
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<tr>
<td>282</td>
<td>Rs12545733</td>
<td>1</td>
<td>1518</td>
<td>0.74</td>
<td>1454</td>
<td>0.71</td>
<td>C</td>
<td>0.019120</td>
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<tr>
<td></td>
<td></td>
<td>2</td>
<td>528</td>
<td>0.26</td>
<td>596</td>
<td>0.29</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 3.2 - Association with single SNPs, genotype statistics test:

a) dominant model risk allele R vs non-risk allele (n)

<table>
<thead>
<tr>
<th>SNP identity</th>
<th>dbSNP reference</th>
<th>Sample</th>
<th>Genotype RR + Rn</th>
<th>Genotype nn</th>
<th>Yates Statistic (df = 1)</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>271</td>
<td>Rs1001793</td>
<td>Cas</td>
<td>483</td>
<td>545</td>
<td>5.07</td>
<td>0.024390</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>533</td>
<td>491</td>
<td></td>
<td></td>
</tr>
<tr>
<td>278</td>
<td>Rs7830593</td>
<td>Cas</td>
<td>438</td>
<td>587</td>
<td>8.16</td>
<td>0.004290</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>373</td>
<td>650</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
b) recessive model homozygous for risk allele R vs Rn + nn

<table>
<thead>
<tr>
<th>SNP identity</th>
<th>dbSNP reference</th>
<th>Sample</th>
<th>Genotype RR</th>
<th>Genotype Rn + nn</th>
<th>Yates Statistic (df = 1)</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>272</td>
<td>Rs12677679</td>
<td>Cas Control</td>
<td>269 570</td>
<td>399 454</td>
<td>6.22</td>
<td>0.012660</td>
</tr>
<tr>
<td>273</td>
<td>Rs10866819</td>
<td>Cas Control</td>
<td>374 655</td>
<td>429 597</td>
<td>6.22</td>
<td>0.012620</td>
</tr>
<tr>
<td>280</td>
<td>Rs4518666</td>
<td>cases</td>
<td>375 648</td>
<td>434 586</td>
<td>7.17</td>
<td>0.007420</td>
</tr>
<tr>
<td>282</td>
<td>Rs12545733</td>
<td>controls cases</td>
<td>66 21</td>
<td>957 929</td>
<td>5.58</td>
<td>0.018510</td>
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</tbody>
</table>

3.3 - Association with haplotypes:

<table>
<thead>
<tr>
<th>SNP used in haplotype</th>
<th>Alleles composing haplotype</th>
<th>Frequency of haplotype in cases</th>
<th>Frequency of haplotype in controls</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>271 – 280</td>
<td>2 – 1</td>
<td>0.343</td>
<td>0.295</td>
<td>6.02 * 10^-4</td>
</tr>
<tr>
<td>271 – 280 – 282</td>
<td>2 – 1 – 1</td>
<td>0.318</td>
<td>0.269</td>
<td>4.21 * 10^-4</td>
</tr>
<tr>
<td>271 – 272 – 280 - 282</td>
<td>2 – 1 – 1 – 1</td>
<td>0.322</td>
<td>0.268</td>
<td>3.03 * 10^-4</td>
</tr>
</tbody>
</table>
REFERENCES


CLAIMS

1. A diagnostic method of determining whether a subject is at risk of developing type 2 diabetes, which method comprises detecting the presence of an alteration in the TNFRSF1OB gene locus in a biological sample of said subject.

2. The method of claim 1, wherein said alteration is one or several SNP(s).

3. The method of claim 2, wherein said SNP is selected from the group consisting of SNP 271, SNP 272, SNP 278, SNP 280, SNP 281, and SNP 282.

4. The method of claim 3, wherein said SNP is allele G of SNP 271.

5. The method of claim 1, wherein said alteration is a haplotype of SNPs which consists in allele G of SNP 271, allele A of SNP 272, allele C of SNP 280 and allele C of SNP 282.

6. The method of any of claims 1-5, wherein the presence of an alteration in the TNFRSF1OB gene locus is detected by sequencing, selective hybridization and/or selective amplification.
**FIGURE**

**Linkage Analysis Results**

![Graph showing linkage analysis results with log(-p-values) on the y-axis and mega base pairs on the x-axis.]