GENETIC VARIANTS IN THE TCF7L2 GENE AS DIAGNOSTIC MARKERS FOR RISK OF TYPE 2 DIABETES MELLITUS

BACKGROUND OF THE INVENTION

Diabetes mellitus, a metabolic disease wherein carbohydrate utilization is reduced and lipid and protein utilization is enhanced, is caused by an absolute or relative deficiency of insulin. In the more severe cases, diabetes is characterized by chronic hyperglycemia, glycosuria, water and electrolyte loss, ketoacidosis and coma. Long term complications include development of neuropathy, retinopathy, nephropathy, generalized degenerative changes in large and small blood vessels and increased susceptibility to infection. The most common form of diabetes is Type II, non-insulin-dependent diabetes that is characterized by hyperglycemia due to impaired insulin secretion and insulin resistance in target tissues. Both genetic and environmental factors contribute to the disease. For example, obesity plays a major role in the development of the disease. Type II diabetes is often a mild form of diabetes mellitus of gradual onset.

The health implications of Type II diabetes are enormous. In 1995, there were 135 million adults with diabetes worldwide. It is estimated that close to 300 million will have diabetes in the year 2025. (King H., *et al., Diabetes Care,* 21(9): 1414-1431 (1998)). The prevalence of Type II diabetes in the adult population in Iceland is 2.5% (Vilbergsson, S., *et al., Diabet. Med.*; 14(6): 491-498 (1997)), which comprises approximately 5,000 people over the age of 34 who have the disease. The high prevalence of the disease and increasing population affected shows an unmet medical need to define the genetic factors involved in Type II diabetes to more precisely define the associated risk factors. Also needed are therapeutic agents for prevention of Type II diabetes.

SUMMARY OF THE INVENTION

The present invention relates to methods of diagnosing an increased susceptibility to type II diabetes, as well as methods of diagnosing a decreased susceptibility to type II diabetes or diagnosing a protection against type II diabetes, by evaluating certain markers or haplotypes relating to the *TCF7L2* gene (transcription factor 7-like 2 (T-cell specific,

HMG-box), previously referred to as the *TCF4* gene (T-cell transcription factor 4)). The methods comprise detecting a genetic marker associated with the exon 4 LD block of *TCF7L2* gene.

In a first aspect, the invention relates to a method of diagnosing a susceptibility to type II diabetes in an individual, comprising analyzing a nucleic acid sample obtained from the individual for a marker or haplotype associated with the exon 4 LD block of TCF7L2, wherein the presence of the marker or haplotype is indicative of a susceptibility to type II diabetes. In one embodiment, the marker or haplotype comprises at least one marker selected from the markers listed in Table 6. In another embodiment, the marker or haplotype is a marker.

In one preferred embodiment, the marker or haplotype is indicative of increased susceptibility of type II diabetes. The increased susceptibility is in one embodiment characterized by a relative risk of at least 1.2, including a relative risk of at least 1.3 and a relative risk of at least 1.4. In one embodiment, the marker is selected from the group consisting of DG10S478, rs12255372, rs7895340, rs11196205, rs7901695, rs7903146, rs12243326, and rs4506565, and wherein the presence of a non-0 allele (e.g., -4, 4, 8, 12, 16, 20, or other non-0 allele) in DG10S478, a T allele in rs12255372; an A allele in rs7895340; a C allele in rs11196205; a C allele in rs7901695; a T allele in rs7903146; a C allele in rs12243326; or an T allele in rs4506565, is indicative of increased susceptibility to type II diabetes. In a preferred embodiment, the marker is selected from the group consisting of DG10S478 and rs7903146, and wherein the presence of a non-0 allele in DG10S478 or a T allele in rs7903146 is indicative of increased susceptibility to type II diabetes. In yet another preferred embodiment, the marker is rs7903146, and wherein the presence of a T allele in rs7903146 is indicative of increased susceptibility to type II diabetes.

In another preferred embodiment, the marker or haplotype is indicative of decreased susceptibility of type II diabetes. The decreased susceptibility is in one embodiment characterized by a relative risk of less than 0.8, including a relative risk of less than 0.7. In one embodiment, the marker is selected from the group consisting of DG10S478, rs12255372, rs7895340, rs11196205, rs7901695, rs7903146, rs12243326, and rs4506565, and wherein the presence of a 0 allele in DG10S478, a G allele in SNP rs12255372; a G allele in rs7895340; a G allele in rs11196205; a T allele in rs7901695; a

C allele in rs7903146; a T allele in rs12243326; or an A allele in rs4506565 is indicative of a decreased susceptibility to type II diabetes. In a preferred embodiment, the marker is DG10S478, and wherein the presence of a 0 allele in DG10S478 is indicative of decreased susceptibility to type II diabetes. In another preferred embodiment, the marker is rs7903146, and wherein the presence of a C allele in rs7903146 is indicative of decreased susceptibility to type II diabetes.

In a second aspect, the present invention relates to a kit for assaying a sample from an individual to detect a susceptibility to type II diabetes, wherein the kit comprises one or more reagents for detecting one or more markers associated with the exon 4 LD block of TCF7L2. In one embodiemnt, the one or more reagents comprise at least one contiguous nucleotide sequence that is completely complementary to a region comprising at least one marker associated with the exon 4 LD block of TCF7L2. In one embodiment, the one or markers is selected from the group consisting of DG10S478, rs12255372, rs7895340, rs11196205, rs7901695, rs7903146, rs12243326, and rs4506565. In a preferred embodiment, the one or more marker is DG10S478 or rs7903146. In another preferred embodiment, the marker is the C allele in rs7903146.

In another aspect, the present invention relates to a method of assessing an individual for probability of response to a TCF7L2 therapeutic agent, comprising: detecting a marker associated with the exon 4 LD block of TCF7L2, wherein the presence of the marker is indicative of a probability of a positive response to a TCF7L2 therapeutic agent. In one embodiment, the marker is selected from the group consisting of DG10S478, rs12255372, rs7895340, rs11196205, rs7901695, rs7903146, rs12243326, and rs4506565. In another embodiment, the marker is marker DG10S478 or marker rs7903146, and wherein the presence of a non-0 allele in DG10S478 or a T allele in rs7903146 is indicative of a probability of a positive response to a TCF7L2 therapeutic agent.

Another aspect ot the invention relates to the use of a TCF7L2 therapeutic agent for the manufacture of a medicament for the treatment of type II diabetes. In one embodiment, the TCF7L2 therapeutic agent is an agent that alters activity in the Wnt signaling pathway or in the cadherin pathway. In another embodiment, the TCF7L2 therapeutic agent is an agent selected from the group set forth in the Agent Table.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other objects, features and advantages of the invention will be apparent from the following more particular description of preferred embodiments of the invention.

FIG. 1 depicts the *TCF7L2* region of interest with respect to linkage disequilibrium (LD) of SNPs in HapMap project Build 16. The 215.9kb gene spans seven LD blocks as indicated by the black arrow schematic (based on NCBI RefSeq) which shows the direction of transcription; exons are indicated, with exon 4 highlighted. DG10S478 is located at 114.46 Mb on chromosome 10 (NCBI Build 34) in intron 3 of the *TCF7L2* gene, within a 74.9kb block that incorporates part of intron 3, the whole of exon 4 and part of intron 4 (herein referred to as the "exon 4 LD block of *TCF7L2*"). The SNP markers are plotted equidistantly rather than according to their physical positions. The figure shows two measures of LD – i.e. D' (upper left part of figure) and r^2 (lower right part).

DETAILED DESCRIPTION OF THE INVENTION

A description of preferred embodiments of the invention follows.

Loci Associated with Type II Diabetes

Type II diabetes is characterized by hyperglycemia, which can occur through mechanisms such as impaired insulin secretion, insulin resistance in peripheral tissues and increased glucose output by the liver. Most type II diabetes patients suffer serious complications of chronic hyperglycemia including nephropathy, neuropathy, retinopathy and accelerated development of cardiovascular disease. The prevalence of type II diabetes worldwide is currently 6% but is projected to rise over the next decade(*1*). This increase in prevalence of type II diabetes is attributed to increasing age of the population and rise in obesity.

There is evidence for a genetic component to the risk of type II diabetes, including prevalence differences between various racial groups(*2, 3*), higher concordance rates among monozygotic than dizygotic twins(*4, 5*) and a sibling relative risk (λ_s) for type II diabetes in European populations of approximately 3.5(*6*).

Two approaches have thus far been used to search for genes associated with type II diabetes. Single nucleotide polymorphisms (SNPs) within candidate genes have been tested for association and have, in general, not been replicated or confer only a modest risk of type II diabetes – the most widely reported being a protective Pro12Ala polymorphism in the peroxisome proliferator activated receptor gamma gene (PPARG2)(*7*) and an at risk polymorphism in the potassium inwardly-rectifying channel, subfamily J, member 11 gene (KIR6.2)(*8*).

Genome-wide linkage scans in families with the common form of type II diabetes have yielded several loci, and the primary focus of international research consortia has been on loci on chromosomes 1, 12 and 20 observed in many populations(*6*). The genes in these loci have yet to be uncovered. However, in Mexican Americans, the calpain 10 (*CAPN10*) gene was isolated out of a locus on chromosome 2q; this represents the only gene for the common form of type II diabetes to date to be identified through positional cloning (*9*). The rare Mendelian forms of type II diabetes, namely maturity-onset diabetes of the young (MODY), have yielded six genes by positional cloning(*6*).

We previously reported genome-wide significant linkage to chromosome 5q for type II diabetes mellitus in the Icelandic population(*10*); in the same study, we also reported suggestive evidence of linkage to 10q and 12q. Linkage to the 10q region has also been observed in Mexican Americans(*11*).

Transcription Factor 7-Like 2 Gene (TCF7L2) Association with Type II Diabetes

The present invention relates to identification of a type II diabetes-associated LD block ("exon 4 LD block of TCF7L2") within the gene encoding T-cell transcription factor 4 (TCF4 - official gene symbol TCF7L2). Several markers within the exon 4 LD block of TCF7L2, including microsatellite DG10S478 and SNP markers rs7903146 and rs12255372, have been found to be associated with type II diabetes. The original observation, first found in an Icelandic cohort, of the association of DG10S478 (P=1.3x10^{-9}; Relative risk =1.45; Population attributable risk =22.7%), has subsequently been replicated in a Danish type II diabetes cohort and a United States Caucasian cohort. DG10S478 is located in intron 3 of the *TCF7L2* gene on 10q25.2 and within a well defined LD block of 74.9kb that encapsulates part of intron 3, the whole of exon 4 and part of intron 4. The *TCF7L2* gene product is a high mobility group (HMG) box-

containing transcription factor that plays a role in the Wnt signaling pathway, also known as the APC3/β-catenin/TCF pathway. TCF7L2 mediates the cell type-specific regulation of proglucagon gene expression (a key player in blood glucose homeostasis) through the Wnt pathway members β-catenin and glycogen synthase kinase-3beta(*12*). In addition, Wnt signaling maintains preadipocytes in an undifferentiated state through inhibition of the adipogenic transcription factors CCAAT/enhancer binding protein alpha (C/EBPalpha) and peroxisome proliferator- activated receptor gamma (PPARgamma)(*13*). When Wnt signaling in preadipocytes is prevented by overexpression of dominant-negative TCF7L2, these cells differentiate into adipocytes(*13*). In addition, it has been reported that the Wnt/β-catenin signaling pathway targets PPARgamma activity through physical interaction with β-catenin and TCF7L2 in colon cancer cells(*14*). The multifunctional β-catenin protein is also important for mediating cell adhesion through its binding of cadherins(*15*).

As a result of this discovery, methods are now available for diagnosis of a susceptibility to type II diabetes, as well as for diagnosis of a decreased susceptibility to type II diabetes and/or a protection against type II diabetes. In preferred embodiments of the invention, diagnostic assays are used to identify the presence of particular alleles, including a 0 allele in marker DG10S478 (associated with a decreased susceptibility to type II diabetes and is an allele that is protective against type II diabetes); a non-0 allele (e.g., -4, 4, 8, 12, 16 or 20, or other allele) in marker DG10S478 (associated with susceptibility to type II diabetes); a G allele in SNP rs12255372 (associated with a decreased susceptibility to type II diabetes and is an allele that is protective against type II diabetes): a T allele in SNP rs12255372 (associated with susceptibility to type II diabetes); a G allele in SNP rs7895340 (associated with a decreased susceptibility to type II diabetes and is an allele that is protective against type II diabetes); an A allele in SNP rs7895340 (associated with susceptibility to type II diabetes); a G allele in SNP rs11196205 (associated with a decreased susceptibility to type II diabetes and is an allele that is protective against type II diabetes); a C allele in SNP rs11196205 (associated with susceptibility to type II diabetes); a T allele in SNP rs7901695 (associated with a decreased susceptibility to type II diabetes and is an allele that is protective against type II diabetes); a C allele in SNP rs7901695 (associated with susceptibility to type II diabetes); a C allele in SNP rs7903146 (associated with a decreased susceptibility to type II diabetes

and is an allele that is protective against type II diabetes); a T allele in SNP rs7903146 (associated with a susceptibility to type II diabetes); a C allele in SNP rs12243326 (associated with a susceptibility to type II diabetes); and an T allele in SNP rs4506565 (associated with a susceptibility to type II diabetes). In additional embodiments of the invention, other markers or SNPs, identified using the methods described herein, can be used for diagnosis of a susceptibility to type II diabetes, and also for diagnosis of a decreased susceptibility to type II diabetes or for identification of an allele that is protective against type II diabetes. The diagnostic assays presented below can be used to identify the presence or absence of these particular alleles.

DIAGNOSTIC ASSAYS

Nucleic acids, probes, primers, and antibodies such as those described herein can be used in a variety of methods of diagnosis of a susceptibility to type II diabetes, as well as in kits (*e.g.*, useful for diagnosis of a susceptibility to type II diabetes). Similarly, the nucleic acids, probes, primers, and antibodies described herein can be used in methods of diagnosis of a decreased susceptibility to type II diabetes, as well as in methods of diagnosis of a protection against type II diabetes, and also in kits). In one aspect, the kit comprises primers that can be used to amplify the markers of interest.

In one aspect of the invention, diagnosis of a susceptibility to type II diabetes is made by detecting a polymorphism in a *TCF7L2* nucleic acid as described herein (e.g., the alleles in marker DG10S478 or in SNP rs12255372, rs7895340, rs11196205, rs7901695, rs7903146, rs12243326, rs4506565). The polymorphism can be a change in a *TCF7L2* nucleic acid, such as the insertion or deletion of a single nucleotide, or of more than one nucleotide, resulting in a frame shift; the change of at least one nucleotide, resulting in a change in the encoded amino acid; the change of at least one nucleotide, resulting in the generation of a premature stop codon; the deletion of several nucleotides, resulting in a deletion of one or more amino acids encoded by the nucleotides; the insertion of one or several nucleotides, such as by unequal recombination or gene conversion, resulting in an interruption of the coding sequence of the gene; duplication of all or a part of the gene; transposition of all or a part of the gene; or rearrangement of all or a part of the gene. More than one such change may be present in a single gene. Such sequence changes cause a difference in the polypeptide encoded by a *TCF7L2* nucleic

acid. For example, if the difference is a frame shift change, the frame shift can result in a change in the encoded amino acids, and/or can result in the generation of a premature stop codon, causing generation of a truncated polypeptide. Alternatively, a polymorphism associated with a disease or condition or a susceptibility to a disease or condition associated with a *TCF7L2* nucleic acid can be a synonymous alteration in one or more nucleotides (*i.e.*, an alteration that does not result in a change in the polypeptide encoded by a *TCF7L2* nucleic acid). Such a polymorphism may alter splicing sites, affect the stability or transport of mRNA, or otherwise affect the transcription or translation of the gene. A *TCF7L2* nucleic acid that has any of the changes or alterations described above is referred to herein as an "altered nucleic acid."

In a first method of diagnosing a susceptibility to type II diabetes, hybridization methods, such as Southern analysis, Northern analysis, or *in situ* hybridizations, can be used (see *Current Protocols in Molecular Biology*, Ausubel, F. *et al.*, eds, John Wiley & Sons, including all supplements through 1999). For example, a biological sample (a "test sample") from a test subject (the "test individual") of genomic DNA, RNA, or cDNA, is obtained from an individual (RNA and cDNA can only be used for exonic markers), such as an individual suspected of having, being susceptible to or predisposed for, or carrying a defect for, type II diabetes. The individual can be an adult, child, or fetus. The test sample can be from any source which contains genomic DNA, such as a blood sample, sample of amniotic fluid, sample of cerebrospinal fluid, or tissue sample from skin, muscle, buccal or conjunctival mucosa, placenta, gastrointestinal tract or other organs. A test sample of DNA from fetal cells or tissue can be obtained by appropriate methods, such as by amniocentesis or chorionic villus sampling. The DNA, RNA, or cDNA sample is then examined to determine whether a polymorphism in a *TCF7L2* nucleic acid is present, and/or to determine which splicing variant(s) encoded by the *TCF7L2* is present. The presence of the polymorphism or splicing variant(s) can be indicated by hybridization of the gene in the genomic DNA, RNA, or cDNA to a nucleic acid probe. A "nucleic acid probe", as used herein, can be a DNA probe or an RNA probe; the nucleic acid probe can contain, for example, at least one polymorphism in a *TCF7L2* nucleic acid and/or contain a nucleic acid encoding a particular splicing variant of a *TCF7L2* nucleic acid. The probe can be any of the nucleic acid molecules described above (*e.g.*, the gene

or nucleic acid, a fragment, a vector comprising the gene or nucleic acid, a probe or primer, etc.).

To diagnose a susceptibility to type II diabetes, a hybridization sample can be formed by contacting the test sample containing a *TCF7L2* nucleic acid with at least one nucleic acid probe. A preferred probe for detecting mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to mRNA or genomic DNA sequences described herein. The nucleic acid probe can be, for example, a full-length nucleic acid molecule, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to appropriate mRNA or genomic DNA. Suitable probes for use in the diagnostic assays of the invention are described above (see *e.g.*, probes and primers discussed under the heading, "Nucleic Acids of the Invention").

The hybridization sample is maintained under conditions that are sufficient to allow specific hybridization of the nucleic acid probe to a *TCF7L2* nucleic acid. "Specific hybridization", as used herein, indicates exact hybridization (*e.g.*, with no mismatches). Specific hybridization can be performed under high stringency conditions or moderate stringency conditions, for example, as described above. In a particularly preferred aspect, the hybridization conditions for specific hybridization are high stringency.

Specific hybridization, if present, is then detected using standard methods. If specific hybridization occurs between the nucleic acid probe and *TCF7L2* nucleic acid in the test sample, then the *TCF7L2* has the polymorphism, or is the splicing variant, that is present in the nucleic acid probe. More than one nucleic acid probe can also be used concurrently in this method. Specific hybridization of any one of the nucleic acid probes is indicative of a polymorphism in the *TCF7L2* nucleic acid, or of the presence of a particular splicing variant encoding the *TCF7L2* nucleic acid and can be diagnostic for a susceptibility to type II diabetes, or for a decreased susceptibility to type II diabetes (or indicative of a protective allele against type II diabetes).

In Northern analysis (see *Current Protocols in Molecular Biology*, Ausubel, F. *et al.*, eds., John Wiley & Sons, *supra*) the hybridization methods described above are used to identify the presence of a polymorphism or a particular splicing variant, associated with a susceptibility to type II diabetes or associated with a decreased susceptibility to

type II diabetes. For Northern analysis, a test sample of RNA is obtained from the individual by appropriate means. Specific hybridization of a nucleic acid probe, as described above, to RNA from the individual is indicative of a polymorphism in a *TCF7L2* nucleic acid, or of the presence of a particular splicing variant encoded by a *TCF7L2* nucleic acid and is therefore diagnostic for the susceptibility to type II diabetes or the decreased susceptibility to type II diabetes (or indicative of a protective allele against type II diabetes).

For representative examples of use of nucleic acid probes, see, for example, U.S. Patents No. 5,288,611 and 4,851,330.

Alternatively, a peptide nucleic acid (PNA) probe can be used instead of a nucleic acid probe in the hybridization methods described above. PNA is a DNA mimic having a peptide-like, inorganic backbone, such as N-(2-aminoethyl) glycine units, with an organic base (A, G, C, T or U) attached to the glycine nitrogen via a methylene carbonyl linker (see, for example, Nielsen, P.E. *et al.*, *Bioconjugate Chemistry* 5, American Chemical Society, p. 1 (1994). The PNA probe can be designed to specifically hybridize to a *TCF7L2* nucleic acid. Hybridization of the PNA probe to a *TCF7L2* nucleic acid can be diagnostic for a susceptibility to type II diabetes or decreased susceptibility to type II diabetes (or indicative of a protective allele against type II diabetes).

In another method of the invention, alteration analysis by restriction digestion can be used to detect an alteration in the gene, if the alteration (mutation) or polymorphism in the gene results in the creation or elimination of a restriction site. A test sample containing genomic DNA is obtained from the individual. Polymerase chain reaction (PCR) can be used to amplify a *TCF7L2* nucleic acid (and, if necessary, the flanking sequences) in the test sample of genomic DNA from the test individual. RFLP analysis is conducted as described (see *Current Protocols in Molecular Biology*, *supra*). The digestion pattern of the relevant DNA fragment indicates the presence or absence of the alteration or polymorphism in the *TCF7L2* nucleic acid, and therefore indicates the presence or absence a susceptibility to type II diabetes or a decreased susceptibility to type II diabetes (or indicative of a protective allele against type II diabetes).

Sequence analysis can also be used to detect specific polymorphisms in a *TCF7L2* nucleic acid. A test sample of DNA or RNA is obtained from the test individual. PCR or other appropriate methods can be used to amplify the gene or nucleic acid, and/or its

flanking sequences, if desired. The sequence of a *TCF7L2* nucleic acid, or a fragment of the nucleic acid, or cDNA, or fragment of the cDNA, or mRNA, or fragment of the mRNA, is determined, using standard methods. The sequence of the nucleic acid, nucleic acid fragment, cDNA, cDNA fragment, mRNA, or mRNA fragment is compared with the known nucleic acid sequence of the gene or cDNA or mRNA, as appropriate. The presence of a polymorphism in the *TCF7L2* indicates that the individual has a susceptibility to type II diabetes or a decreased susceptibility to type II diabetes (or indicative of a protective allele against type II diabetes).

Allele-specific oligonucleotides can also be used to detect the presence of a polymorphism in a *TCF7L2* nucleic acid, through the use of dot-blot hybridization of amplified oligonucleotides with allele-specific oligonucleotide (ASO) probes (see, for example, Saiki, R. *et al.*, *Nature* 324:163-166 (1986)). An "allele-specific oligonucleotide" (also referred to herein as an "allele-specific oligonucleotide probe") is an oligonucleotide of approximately 10-50 base pairs, preferably approximately 15-30 base pairs, that specifically hybridizes to a *TCF7L2* nucleic acid, and that contains a polymorphism associated with a susceptibility to type II diabetes or a polymorphism associated with a decreased susceptibility to type II diabetes (or indicative of a protective allele against type II diabetes). An allele-specific oligonucleotide probe that is specific for particular polymorphisms in a *TCF7L2* nucleic acid can be prepared, using standard methods (see *Current Protocols in Molecular Biology*, *supra*). To identify polymorphisms in the gene that are associated with type II diabetes, a test sample of DNA is obtained from the individual. PCR can be used to amplify all or a fragment of a *TCF7L2* nucleic acid and its flanking sequences. The DNA containing the amplified *TCF7L2* nucleic acid (or fragment of the gene or nucleic acid) is dot-blotted, using standard methods (see *Current Protocols in Molecular Biology*, *supra*), and the blot is contacted with the oligonucleotide probe. The presence of specific hybridization of the probe to the amplified *TCF7L2* nucleic acid is then detected. Hybridization of an allele-specific oligonucleotide probe to DNA from the individual is indicative of a polymorphism in the *TCF7L2* nucleic acid, and is therefore indicative of susceptibility to type II diabetes or is indicative of decreased susceptibility to type II diabetes (or indicative of a protective allele against type II diabetes).

The invention further provides allele-specific oligonucleotides that hybridize to the reference or variant allele of a gene or nucleic acid comprising a single nucleotide polymorphism or to the complement thereof. These oligonucleotides can be probes or primers.

An allele-specific primer hybridizes to a site on target DNA overlapping a polymorphism and only primes amplification of an allelic form to which the primer exhibits perfect complementarity. See Gibbs, *Nucleic Acid Res.* 17, 2427-2448 (1989). This primer is used in conjunction with a second primer, which hybridizes at a distal site. Amplification proceeds from the two primers, resulting in a detectable product, which indicates the particular allelic form is present. A control is usually performed with a second pair of primers, one of which shows a single base mismatch at the polymorphic site and the other of which exhibits perfect complementarity to a distal site. The single-base mismatch prevents amplification and no detectable product is formed. The method works best when the mismatch is included in the 3'-most position of the oligonucleotide aligned with the polymorphism because this position is most destabilizing to elongation from the primer (see, *e.g.*, WO 93/22456).

With the addition of such analogs as locked nucleic acids (LNAs), the size of primers and probes can be reduced to as few as 8 bases. LNAs are a novel class of bicyclic DNA analogs in which the 2' and 4' positions in the furanose ring are joined via an O-methylene (oxy-LNA), S-methylene (thio-LNA), or amino methylene (amino-LNA) moiety. Common to all of these LNA variants is an affinity toward complementary nucleic acids, which is by far the highest reported for a DNA analog. For example, particular all oxy-LNA nonamers have been shown to have melting temperatures of 64EC and 74EC when in complex with complementary DNA or RNA, respectively, as opposed to 28EC for both DNA and RNA for the corresponding DNA nonamer. Substantial increases in T_m are also obtained when LNA monomers are used in combination with standard DNA or RNA monomers. For primers and probes, depending on where the LNA monomers are included (*e.g.*, the 3' end, the 5'end, or in the middle), the T_m could be increased considerably.

In another aspect, arrays of oligonucleotide probes that are complementary to target nucleic acid sequence segments from an individual can be used to identify polymorphisms in a *TCF7L2* nucleic acid. For example, in one aspect, an oligonucleotide

array can be used. Oligonucleotide arrays typically comprise a plurality of different oligonucleotide probes that are coupled to a surface of a substrate in different known locations. These oligonucleotide arrays, also described as "Genechips™," have been generally described in the art, for example, U.S. Pat. No. 5,143,854 and PCT patent publication Nos. WO 90/15070 and 92/10092. These arrays can generally be produced using mechanical synthesis methods or light directed synthesis methods that incorporate a combination of photolithographic methods and solid phase oligonucleotide synthesis methods. See Fodor *et al.*, *Science* 251:767-777 (1991), Pirrung *et al.*, U.S. Pat. No. 5,143,854 (see also PCT Application No. WO 90/15070) and Fodor *et al.*, PCT Publication No. WO 92/10092 and U.S. Pat. No. 5,424,186, the entire teachings are incorporated by reference herein. Techniques for the synthesis of these arrays using mechanical synthesis methods are described in, *e.g.*, U.S. Pat. No. 5,384,261; the entire teachings are incorporated by reference herein. In another example, linear arrays can be utilized.

Once an oligonucleotide array is prepared, a nucleic acid of interest is hybridized with the array and scanned for polymorphisms. Hybridization and scanning are generally carried out by methods described herein and also in, *e.g.*, published PCT Application Nos. WO 92/10092 and WO 95/11995, and U.S. Pat. No. 5,424,186, the entire teachings are incorporated by reference herein. In brief, a target nucleic acid sequence that includes one or more previously identified polymorphic markers is amplified by well-known amplification techniques, *e.g.*, PCR. Typically, this involves the use of primer sequences that are complementary to the two strands of the target sequence both upstream and downstream from the polymorphism. Asymmetric PCR techniques may also be used. Amplified target, generally incorporating a label, is then hybridized with the array under appropriate conditions. Upon completion of hybridization and washing of the array, the array is scanned to determine the position on the array to which the target sequence hybridizes. The hybridization data obtained from the scan is typically in the form of fluorescence intensities as a function of location on the array.

Although primarily described in terms of a single detection block, *e.g.*, for detecting a single polymorphism, arrays can include multiple detection blocks, and thus be capable of analyzing multiple, specific polymorphisms. In alternative aspects, it will generally be understood that detection blocks may be grouped within a single array or in

multiple, separate arrays so that varying, optimal conditions may be used during the hybridization of the target to the array. For example, it may often be desirable to provide for the detection of those polymorphisms that fall within G-C rich stretches of a genomic sequence, separately from those falling in A-T rich segments. This allows for the separate optimization of hybridization conditions for each situation.

Additional uses of oligonucleotide arrays for polymorphism detection can be found, for example, in U.S. Patents Nos. 5,858,659 and 5,837,832, the entire teachings of which are incorporated by reference herein. Other methods of nucleic acid analysis can be used to detect polymorphisms in a type II diabetes gene or variants encoded by a type II diabetes gene. Representative methods include direct manual sequencing (Church and Gilbert, *Proc. Natl. Acad. Sci. USA* 81:1991-1995 (1988); Sanger, F. *et al.*, *Proc. Natl. Acad. Sci. USA* 74:5463-5467 (1977); Beavis *et al.*, U.S. Pat. No. 5,288,644); automated fluorescent sequencing; single-stranded conformation polymorphism assays (SSCP); clamped denaturing gel electrophoresis (CDGE); denaturing gradient gel electrophoresis (DGGE) (Sheffield, V.C. *et al.*, *Proc. Natl. Acad. Sci. USA* 86:232-236 (1989)), mobility shift analysis (Orita, M. *et al.*, *Proc. Natl. Acad. Sci. USA* 86:2766-2770 (1989)), restriction enzyme analysis (Flavell *et al.*, *Cell* 15:25 (1978); Geever, *et al.*, *Proc. Natl. Acad. Sci. USA* 78:5081 (1981)); heteroduplex analysis; chemical mismatch cleavage (CMC) (Cotton *et al.*, *Proc. Natl. Acad. Sci. USA* 85:4397-4401 (1985)); RNase protection assays (Myers, R.M. *et al.*, *Science* 230:1242 (1985)); use of polypeptides which recognize nucleotide mismatches, such as *E. coli* mutS protein; allele-specific PCR, for example.

In one aspect of the invention, diagnosis of a susceptibility to type II diabetes, or of a decreased susceptibility to type II diabetes (or indicative of a protective allele against type II diabetes), can also be made by expression analysis by quantitative PCR (kinetic thermal cycling). This technique, utilizing TaqMan® assays, can assess the presence of an alteration in the expression or composition of the polypeptide encoded by a *TCF7L2* nucleic acid or splicing variants encoded by a *TCF7L2* nucleic acid. TaqMan® probes can also be used to allow the identification of polymorphisms and whether a patient is homozygous or heterozygous. Further, the expression of the variants can be quantified as physically or functionally different.

In another aspect of the invention, diagnosis of a susceptibility to type II diabetes or of a decreased susceptibility to type II diabetes (or indicative of a protective allele against type II diabetes), can be made by examining expression and/or composition of a TCF7L2 polypeptide, by a variety of methods, including enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. A test sample from an individual is assessed for the presence of an alteration in the expression and/or an alteration in composition of the polypeptide encoded by a *TCF7L2* nucleic acid, or for the presence of a particular variant encoded by a *TCF7L2* nucleic acid. An alteration in expression of a polypeptide encoded by a *TCF7L2* nucleic acid can be, for example, an alteration in the quantitative polypeptide expression (*i.e.*, the amount of polypeptide produced); an alteration in the composition of a polypeptide encoded by a *TCF7L2* nucleic acid is an alteration in the qualitative polypeptide expression (*e.g.*, expression of an altered TCF7L2 polypeptide or of a different splicing variant). In a preferred aspect, diagnosis of a susceptibility to type II diabetes or of a decreased susceptibility to type II diabetes can be made by detecting a particular splicing variant encoded by that *TCF7L2* nucleic acid, or a particular pattern of splicing variants.

Both such alterations (quantitative and qualitative) can also be present. The term "alteration" in the polypeptide expression or composition, as used herein, refers to an alteration in expression or composition in a test sample, as compared with the expression or composition of polypeptide by a *TCF7L2* nucleic acid in a control sample. A control sample is a sample that corresponds to the test sample (*e.g.*, is from the same type of cells), and is from an individual who is not affected by a susceptibility to type II diabetes. An alteration in the expression or composition of the polypeptide in the test sample, as compared with the control sample, is indicative of a susceptibility to type II diabetes. Similarly, the presence of one or more different splicing variants in the test sample, or the presence of significantly different amounts of different splicing variants in the test sample, as compared with the control sample, is indicative of a susceptibility to type II diabetes. Various means of examining expression or composition of the polypeptide encoded by a *TCF7L2* nucleic acid can be used, including: spectroscopy, colorimetry, electrophoresis, isoelectric focusing, and immunoassays (*e.g.*, David *et al.*, U.S. Pat. 4,376,110) such as immunoblotting (see also *Current Protocols in Molecular Biology*, particularly Chapter 10). For example, in one aspect, an antibody capable of binding to

the polypeptide (*e.g.*, as described above), preferably an antibody with a detectable label, can be used. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or $F(ab')_2$) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin.

Western blotting analysis, using an antibody as described above that specifically binds to a polypeptide encoded by an altered *TCF7L2* nucleic acid or an antibody that specifically binds to a polypeptide encoded by a non-altered nucleic acid, or an antibody that specifically binds to a particular splicing variant encoded by a nucleic acid, can be used to identify the presence in a test sample of a particular splicing variant or of a polypeptide encoded by a polymorphic or altered *TCF7L2* nucleic acid, or the absence in a test sample of a particular splicing variant or of a polypeptide encoded by a non-polymorphic or non-altered nucleic acid. The presence of a polypeptide encoded by a polymorphic or altered nucleic acid, or the absence of a polypeptide encoded by a non-polymorphic or non-altered nucleic acid, is diagnostic for a susceptibility to type II diabetes, as is the presence (or absence) of particular splicing variants encoded by the *TCF7L2* nucleic acid.

In one aspect of this method, the level or amount of polypeptide encoded by a *TCF7L2* nucleic acid in a test sample is compared with the level or amount of the polypeptide encoded by the TCF7L2 in a control sample. A level or amount of the polypeptide in the test sample that is higher or lower than the level or amount of the polypeptide in the control sample, such that the difference is statistically significant, is indicative of an alteration in the expression of the polypeptide encoded by the *TCF7L2* nucleic acid, and is diagnostic for a susceptibility to type II diabetes. Alternatively, the composition of the polypeptide encoded by a *TCF7L2* nucleic acid in a test sample is compared with the composition of the polypeptide encoded by the *TCF7L2* nucleic acid in a control sample (*e.g.*, the presence of different splicing variants). A difference in the

composition of the polypeptide in the test sample, as compared with the composition of the polypeptide in the control sample, is diagnostic for a susceptibility to type II diabetes. In another aspect, both the level or amount and the composition of the polypeptide can be assessed in the test sample and in the control sample. A difference in the amount or level of the polypeptide in the test sample, compared to the control sample; a difference in composition in the test sample, compared to the control sample; or both a difference in the amount or level, and a difference in the composition, is indicative of a susceptibility to type II diabetes.

The same methods can conversely be used to identify the presence of a difference when compared to a control (disease) sample. A difference from the control is indicative of a decreased susceptibility to diabetes, and/or is indicative of a protective allele against type II diabetes.

ASSESSMENT FOR MARKERS AND HAPLOTYPES

Populations of individuals exhibiting genetic diversity do not have identical genomes. Rather, the genome exhibits sequence variability between individuals at many locations in the genome; in other words, there are many polymorphic sites in a population. In some instances, reference is made to different alleles at a polymorphic site without choosing a reference allele. Alternatively, a reference sequence can be referred to for a particular polymorphic site. The reference allele is sometimes referred to as the "wild-type" allele and it usually is chosen as either the first sequenced allele or as the allele from a "non-affected" individual (*e.g.*, an individual that does not display a disease or abnormal phenotype). Alleles that differ from the reference are referred to as "variant" alleles.

A "marker", as described herein, refers to a genomic sequence characteristic of a particular variant allele (i.e. polymorphic site). The marker can comprise any allele of any variant type found in the genome, including SNPs, microsatellites, insertions, deletions, duplications and translocations.

SNP nomenclature as reported herein refers to the official Reference SNP (rs) ID identification tag as assigned to each unique SNP by the National Center for Biotechnological Information (NCBI).

A "haplotype," as described herein, refers to a segment of a genomic DNA strand that is characterized by a specific combination of genetic markers ("alleles") arranged along the segment. In a certain embodiment, the haplotype can comprise one or more alleles, two or more alleles, three or more alleles, four or more alleles, or five or more alleles. The genetic markers are particular "alleles" at "polymorphic sites" associated with the exon 4 LD block of TCF7L2. As used herein, "exon 4 LD block of TCF7L2" refers to the LD block on Chr10q whithin which association of variants to type II diabetes is observed. NCBI Build 34 position of this LD block is from 114,413,084 – 114,488,013 bp. The term "susceptibility", as described herein, encompasses both increased susceptibility and decreased susceptibility. Thus, particular markers and/or haplotypes of the invention may be characteristic of increased susceptility of type II diabetes, as characterized by a relative risk of greater than one. Markers and/or haplotypes that confer increased susceptibility of type II diabetes are furthermore considered to be "at-risk", as they confer an increased risk of disease. Alternatively, the markers and/or haplotypes of the invention are characteristic of decreased susceptibility of type II diabetes, as characterized by a relative risk of less than one.

A nucleotide position at which more than one sequence is possible in a population (either a natural population or a synthetic population, *e.g.*, a library of synthetic molecules) is referred to herein as a "polymorphic site". Where a polymorphic site is a single nucleotide in length, the site is referred to as a single nucleotide polymorphism ("SNP"). For example, if at a particular chromosomal location, one member of a population has an adenine and another member of the population has a thymine at the same position, then this position is a polymorphic site, and, more specifically, the polymorphic site is a SNP. Alleles for SNP markers as referred to herein refer to the bases A, C, G or T as they occur at the polymorphic site in the SNP assay employed. The person skilled in the art will realise that by assaying or reading the opposite strand, the complementary allele can in each case be measured. Thus, for a polymorphic site containing an A/G polymorphism, the assay employed may either measure the percentage or ratio of the two bases possible, i.e. A and G. Alternatively, by designing an assay that determines the opposite strand on the DNA template, the percentage or ratio of the complementary bases T/C can be measured. Quantitatively (for example, in terms of relative risk), identical results would be obtained from measurement of either DNA strand

(+ strand or – strand). Polymorphic sites can allow for differences in sequences based on substitutions, insertions or deletions. For example, a polymorphic microsatellite has multiple small repeats of bases (such as CA repeats) at a particular site in which the number of repeat lengths varies in the general population. Each version of the sequence with respect to the polymorphic site is referred to herein as an "allele" of the polymorphic site. Thus, in the previous example, the SNP allows for both an adenine allele and a thymine allele. SNPs and microsatellite markers located within the exon 4 LD block of TCF7L2 found to be associated with type II diabes are described in Tables 2 – 7.

Typically, a reference sequence is referred to for a particular sequence. Alleles that differ from the reference are referred to as "variant" alleles. For example, the reference genomic DNA sequence between positions 114413084 and 114488013 of NCBI Build 34 (equals 74929 bp, or 74.9kb), which refers to the location within Chromosome 10, is described herein as SEQ ID NO:1. A variant sequence, as used herein, refers to a sequence that differs from SEQ ID NO:1 but is otherwise substantially similar. The genetic markers that make up the haplotypes associated with the exon 4 LD block of TCF7L2 are variants. Additional variants can include changes that affect a polypeptide, *e.g.*, a polypeptide encoded by the *TCF7L2* gene. These sequence differences, when compared to a reference nucleotide sequence, can include the insertion or deletion of a single nucleotide, or of more than one nucleotide. Such sequence differences may result in a frame shift; the change of at least one nucleotide, may result in a change in the encoded amino acid; the change of at least one nucleotide, may result in the generation of a premature stop codon; the deletion of several nucleotides, may result in a deletion of one or more amino acids encoded by the nucleotides; the insertion of one or several nucleotides, such as by unequal recombination or gene conversion, may result in an interruption of the coding sequence of a reading frame; duplication of all or a part of a sequence; transposition; or a rearrangement of a nucleotide sequence, as described in detail herein. Such sequence changes alter the polypeptide encoded by the nucleic acid. For example, if the change in the nucleic acid sequence causes a frame shift, the frame shift can result in a change in the encoded amino acids, and/or can result in the generation of a premature stop codon, causing generation of a truncated polypeptide. Alternatively, a polymorphism associated with type II diabetes or a susceptibility to type II diabetes can be a synonymous change in one or more nucleotides (*i.e.*, a change that does not result in

a change in the amino acid sequence). Such a polymorphism can, for example, alter splice sites, affect the stability or transport of mRNA, or otherwise affect the transcription or translation of an encoded polypeptide. It can also alter DNA to increase the possibility that structural changes, such as amplifications or deletions, occur at the somatic level in tumors. The polypeptide encoded by the reference nucleotide sequence is the "reference" polypeptide with a particular reference amino acid sequence, and polypeptides encoded by variant alleles are referred to as "variant" polypeptides with variant amino acid sequences.

A polymorphic microsatellite has multiple small repeats of bases that are 2-8 nucleotides in length (such as CA repeats) at a particular site, in which the number of repeat lengths varies in the general population. An indel is a common form of polymorphism comprising a small insertion or deletion that is typically only a few nucleotides long.

The haplotypes described herein are a combination of various genetic markers, *e.g.*, SNPs and microsatellites, having particular alleles at polymorphic sites. The haplotypes can comprise a combination of various genetic markers, therefore, detecting haplotypes can be accomplished by methods known in the art for detecting sequences at polymorphic sites. For example, standard techniques for genotyping for the presence of SNPs and/or microsatellite markers can be used, such as fluorescence-based techniques (Chen, X. *et al.*, *Genome Res. 9(5)*: 492-98 (1999)), PCR, LCR, Nested PCR and other techniques for nucleic acid amplification. These markers and SNPs can be identified in at-risk haplotypes. Certain methods of identifying relevant markers and SNPs include the use of linkage disequilibrium (LD) and/or LOD scores.

In certain methods described herein, an individual who is at-risk for type II diabetes is an individual in whom an at-risk marker or haplotype is identified. In one aspect, the at-risk marker or haplotype is one that confers a significant increased risk (or susceptility) of type II diabetes. In one embodiment, significance associated with a marker or haplotype is measured by a relative risk. In a further embodiment, the significance is measured by a percentage. In one embodiment, a significant increased risk is measured as a relative risk of at least about 1.2, including but not limited to: 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8 and 1.9. In a further embodiment, a relative risk of at least 1.2 is significant. In a further embodiment, a relative risk of at least about 1.5 is significant. In a further embodiment, a significant increase in risk

is at least about 1.7 is significant. In a further embodiment, a significant increase in risk is at least about 20%, including but not limited to about 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% and 98%. In a further embodiment, a significant increase in risk is at least about 50%.

In other embodiments of the invention, the marker or haplotype confers decreased risk (decreased susceptibility) of type II diabetes. In one embodiment, significant decreased risk is measured as a relative risk at less than 0.9, including but not limited to 0.9, 0.8, 0.7, 0.6, 0.5, and 0.4. In a further embodiment, significant relative risk is less than 0.7. In another embodiment, the decreased in risk (or susceptibility) is at least about 20%, including but not limited to about 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% and 98%. In a further embodiment, a significant decrease in risk is at least about 30%.

Thus, the term "susceptibility to type II diabetes" indicates either an increased risk or susceptility or a decreased risk or susceptibility of type II diabetes, by an amount that is significant, when a certain allele, marker, SNP or haplotype is present; significance is measured as indicated above. The terms "decreased risk", "decreased susceptibility" and "protection against," as used herein, indicate that the relative risk is decreased accordingly when a certain other allele, marker, SNP, and/or a certain other haplotype, is present. It is understood however, that identifying whether an increased or decreased risk is medically significant may also depend on a variety of factors, including the specific disease, the marker or haplotype, and often, environmental factors.

An at-risk marker or haplotype in, or comprising portions of, the *TCF7L2* gene, is one where the marker or haplotype is more frequently present in an individual at risk for type II diabetes (affected), compared to the frequency of its presence in a healthy individual (control), and wherein the presence of the marker or haplotype is indicative of susceptibility to type II diabetes. As an example of a simple test for correlation would be a Fisher-exact test on a two by two table. Given a cohort of chromosomes the two by two table is constructed out of the number of chromosomes that include both of the markers or haplotypes, one of the markers or haplotypes but not the other and neither of the markers or haplotypes.

In certain aspects of the invention, at-risk marker or haplotype is an at-risk marker or haplotype within or near *TCF7L2* that significantly correlates with type II diabetes. In

other aspects, an at-risk marker or haplotype comprises an at-risk marker or haplotype within or near *TCF7L2* that significantly correlates with susceptibility to type II diabetes. In particular embodiments of the invention, the marker or haplotype is associated with the exon 4 LD block of *TCF7L2*, as described herein.

Standard techniques for genotyping for the presence of SNPs and/or microsatellite markers can be used, such as fluorescent based techniques (Chen, *et al.*, *Genome Res. 9*, 492 (1999)), PCR, LCR, Nested PCR and other techniques for nucleic acid amplification. In a preferred aspect, the method comprises assessing in an individual the presence or frequency of SNPs and/or microsatellites in, comprising portions of, the *TCF7L2* gene, wherein an excess or higher frequency of the SNPs and/or microsatellites compared to a healthy control individual is indicative that the individual is susceptible to type II diabetes. Such SNPs and markers can form haplotypes that can be used as screening tools. These markers and SNPs can be identified in at-risk haploptypes. For example, an at-risk haplotype can include microsatellite markers and/or SNPs such as marker DG10S478 and/or SNP rs12255372, rs7895340, rs11196205, rs7901695, rs7903146, rs12243326 or rs4506565. The presence of an at-risk haplotype is indicative of increased susceptibility to type II diabetes, and therefore is indicative of an individual who falls within a target population for the treatment methods described herein.

Identification of susceptibility variants

The frequencies of haplotypes in the patient and the control groups can be estimated using an expectation-maximization algorithm (Dempster A. *et al.*, *J. R. Stat. Soc. B*, 39:1-38 (1977)). An implementation of this algorithm that can handle missing genotypes and uncertainty with the phase can be used. Under the null hypothesis, the patients and the controls are assumed to have identical frequencies. Using a likelihood approach, an alternative hypothesis is tested, where a candidate at-risk-haplotype, which can include the markers described herein, is allowed to have a higher frequency in patients than controls, while the ratios of the frequencies of other haplotypes are assumed to be the same in both groups. Likelihoods are maximized separately under both hypotheses and a corresponding 1-df likelihood ratio statistic is used to evaluate the statistical significance.

To look for at-risk and protective markers and haplotypes within a linkage region, for example, association of all possible combinations of genotyped markers is studied, provided those markers span a practical region. The combined patient and control groups can be randomly divided into two sets, equal in size to the original group of patients and controls. The marker and haplotype analysis is then repeated and the most significant p-value registered is determined. This randomization scheme can be repeated, for example, over 100 times to construct an empirical distribution of p-values. In a preferred embodiment, a p-value of <0.05 is indicative of an significant marker and/or haplotype association.

A detailed discussion of haplotype analysis follows.

Haplotype Analysis

One general approach to haplotype analysis involves using likelihood-based inference applied to NEsted MOdels (Gretarsdottir S., *et al.*, *Nat. Genet. 35*:131-38 (2003)). The method is implemented in the program NEMO, which allows for many polymorphic markers, SNPs and microsatellites. The method and software are specifically designed for case-control studies where the purpose is to identify haplotype groups that confer different risks. It is also a tool for studying LD structures. In NEMO, maximum likelihood estimates, likelihood ratios and p-values are calculated directly, with the aid of the EM algorithm, for the observed data treating it as a missing-data problem.

Measuring Information

Even though likelihood ratio tests based on likelihoods computed directly for the observed data, which have captured the information loss due to uncertainty in phase and missing genotypes, can be relied on to give valid p-values, it would still be of interest to know how much information had been lost due to the information being incomplete. The information measure for haplotype analysis is described in Nicolae and Kong (Technical Report 537, Department of Statistics, University of Statistics, University of Chicago; *Biometrics*, 60(2):368-75 (2004)) as a natural extension of information measures defined for linkage analysis, and is implemented in NEMO.

Statistical Analysis

For single marker association to the disease, the Fisher exact test can be used to calculate two-sided p-values for each individual allele. All p-values are presented unadjusted for multiple comparisons unless specifically indicated. The presented frequencies (for microsatellites, SNPs and haplotypes) are allelic frequencies as opposed to carrier frequencies. To minimize any bias due the relatedness of the patients who were recruited as families for the linkage analysis, first and second-degree relatives can be eliminated from the patient list. Furthermore, the test can be repeated for association correcting for any remaining relatedness among the patients, by extending a variance adjustment procedure described in Risch, N. & Teng, J. (*Genome Res.*, 8:1273-1288 (1998)), DNA pooling (*ibid*) for sibships so that it can be applied to general familial relationships, and present both adjusted and unadjusted p-values for comparison. The differences are in general very small as expected. To assess the significance of single-marker association corrected for multiple testing we can carry out a randomization test using the same genotype data. Cohorts of patients and controls can be randomized and the association analysis redone multiple times (*e.g.*, up to 500,000 times) and the p-value is the fraction of replications that produced a p-value for some marker allele that is lower than or equal to the p-value we observed using the original patient and control cohorts.

For both single-marker and haplotype analyses, relative risk (RR) and the population attributable risk (PAR) can be calculated assuming a multiplicative model (haplotype relative risk model) (Terwilliger, J.D. & Ott, J., *Hum. Hered. 42*:337-46 (1992) and Falk, C.T. & Rubinstein, P, *Ann. Hum. Genet. 51 (Pt 3)*:227-33 (1987)), i.e., that the risks of the two alleles/haplotypes a person carries multiply. For example, if RR is the risk of A relative to a, then the risk of a person homozygote AA will be RR times that of a heterozygote Aa and RR^2 times that of a homozygote aa. The multiplicative model has a nice property that simplifies analysis and computations — haplotypes are independent, *i.e.*, in Hardy-Weinberg equilibrium, within the affected population as well as within the control population. As a consequence, haplotype counts of the affecteds and controls each have multinomial distributions, but with different haplotype frequencies under the alternative hypothesis. Specifically, for two haplotypes, h_i and h_j, $\text{risk}(h_i)/\text{risk}(h_j) = (f_i/p_i)/(f_j/p_j)$, where f and p denote, respectively, frequencies in the affected population and in the control population. While there is some power loss if the

true model is not multiplicative, the loss tends to be mild except for extreme cases. Most importantly, p-values are always valid since they are computed with respect to null hypothesis.

Linkage Disequilibrium Using NEMO

LD between pairs of markers can be calculated using the standard definition of D' and R^2 (Lewontin, R., *Genetics 49*:49-67 (1964); Hill, W.G. & Robertson, A. *Theor. Appl. Genet. 22*:226-231 (1968)). Using NEMO, frequencies of the two marker allele combinations are estimated by maximum likelihood and deviation from linkage equilibrium is evaluated by a likelihood ratio test. The definitions of D' and R^2 are extended to include microsatellites by averaging over the values for all possible allele combination of the two markers weighted by the marginal allele probabilities. When plotting all marker combination to elucidate the LD structure in a particular region, we plot D' in the upper left corner and the p-value in the lower right corner. In the LD plots the markers can be plotted equidistant rather than according to their physical location, if desired.

Statistical Methods for Linkage Analysis

Multipoint, affected-only allele-sharing methods can be used in the analyses to assess evidence for linkage. Results, both the LOD-score and the non-parametric linkage (NPL) score, can be obtained using the program Allegro (Gudbjartsson *et al., Nat. Genet. 25*:12-3 (2000)). Our baseline linkage analysis uses the S_{pairs} scoring function (Whittemore, A.S., Halpern, J. *Biometrics 50*:118-27 (1994); Kruglyak L. *et al., Am. J. Hum. Genet. 58*:1347-63 (1996)), the exponential allele-sharing model (Kong, A. and Cox, N.J., *Am. J. Hum. Genet. 61*:1179-88 (1997)) and a family weighting scheme that is halfway, on the log-scale, between weighting each affected pair equally and weighting each family equally. The information measure that we use is part of the Allegro program output and the information value equals zero if the marker genotypes are completely uninformative and equals one if the genotypes determine the exact amount of allele sharing by decent among the affected relatives (Gretarsdottir *et al., Am. J. Hum. Genet., 70*:593-603 (2002)). The P-values were computed two different ways and the less significant result is reported here. The first P-value can be computed on the basis of large

sample theory; the distribution of $Z_{lr} = \square(2[\log_e(10)LOD])$ approximates a standard normal variable under the null hypothesis of no linkage (Kong, A. and Cox, N.J., *Am. J. Hum. Genet. 61*:1179-88 (1997)). The second P-value can be calculated by comparing the observed LOD-score with its complete data sampling distribution under the null hypothesis (*e.g.*, Gudbjartsson *et al., Nat. Genet. 25:*12-3 (2000)). When the data consist of more than a few families, these two P-values tend to be very similar.

Haplotypes and "Haplotype Block" Definition of a Susceptibility Locus

In certain embodiments, marker and haplotype analysis involves defining a candidate susceptibility locus based on "haplotype blocks" (also called "LD blocks"). It has been reported that portions of the human genome can be broken into series of discrete haplotype blocks containing a few common haplotypes; for these blocks, linkage disequilibrium data provided little evidence indicating recombination (see, *e.g.*, Wall., J.D. and Pritchard, J.K., *Nature Reviews Genetics 4*:587-597 (2003); Daly, M. *et al., Nature Genet. 29*:229-232 (2001); Gabriel, S.B. *et al., Science 296*:2225-2229 (2002); Patil, N. *et al., Science 294*:1719-1723 (2001); Dawson, E. *et al., Nature 418*:544-548 (2002); Phillips, M.S. *et al., Nature Genet. 33*:382-387 (2003)).

There are two main methods for defining these haplotype blocks: blocks can be defined as regions of DNA that have limited haplotype diversity (see, *e.g.*, Daly, M. *et al., Nature Genet. 29*:229-232 (2001); Patil, N. *et al., Science 294*:1719-1723 (2001); Dawson, E. *et al., Nature 418*:544-548 (2002); Zhang, K. *et al., Proc. Natl. Acad. Sci. USA 99*:7335-7339 (2002)), or as regions between transition zones having extensive historical recombination, identified using linkage disequilibrium (see, *e.g.*, Gabriel, S.B. *et al., Science 296*:2225-2229 (2002); Phillips, M.S. *et al., Nature Genet. 33*:382-387 (2003); Wang, N. *et al., Am. J. Hum. Genet. 71*:1227-1234 (2002); Stumpf, M.P., and Goldstein, D.B., *Curr. Biol. 13*:1-8 (2003)). As used herein, the terms "haplotype block" or "LD block" includes blocks defined by either characteristic.

Representative methods for identification of haplotype blocks are set forth, for example, in U.S. Published Patent Application Nos. 20030099964, 20030170665, 20040023237 and 20040146870. Haplotype blocks can be used readily to map associations between phenotype and haplotype status. The main haplotypes can be identified in each haplotype block, and then a set of "tagging" SNPs or markers (the

smallest set of SNPs or markers needed to distinguish among the haplotypes) can then be identified. These tagging SNPs or markers can then be used in assessment of samples from groups of individuals, in order to identify association between phenotype and haplotype. If desired, neighboring haplotype blocks can be assessed concurrently, as there may also exist linkage disequilibrium among the haplotype blocks.

Haplotypes and Diagnostics

As described herein, certain markers and haplotypes comprising such markers are found to be useful for determination of susceptibility to type II diabetes – i.e., they are found to be useful for diagnosing a susceptibility to type II diabetes. Particular markers and haplotypes are found more frequently in individuals with type II diabetes than in individuals without type II diabetes. Therefore, these markers and haplotypes have predictive value for detecting type II diabetes, or a susceptibility to type II diabetes, in an individual. Haplotype blocks (i.e. the exon 4 LD block of TCF7L2) comprising certain tagging markers, can be found more frequently in individuals with type II diabetes than in individuals without type II diabetes. Therefore, these "at-risk" tagging markers within the haplotype block also have predictive value for detecting type II diabes, or a susceptibility to type II diabetes, in an individual. "At-risk" tagging markers within the haplotype or LD blocks can also include other markers that distinguish among the haplotypes, as these similarly have predictive value for detecting type II diabetes or a susceptibility to type II diabetes. As a consequence of the haplotype block structure of the human genome, a large number of markers or other variants and/or haplotypes comprising such markers or variants in association with the haplotype block (LD block) may be found to be associated with a certain trait and/or phenotype. Thus, it is possible that markers and/or haplotypes residing within the exon 4 LD block of TCF7L2 as defined herein or in strong LD (characterized by r^2 greater than 0.2) with the exon 4 LD block of TCF7L2 are associated with type II diabetes (i.e. they confer increased or decreased susceptibility of type II diabetes). This includes markers that are described herein (Table 6), but may also include other markers that are in strong LD (characterized by r^2 greater than 0.2) with one or more of the markers listed in Table 6. The identification of such additional variants can be achieved by methods well known to those skilled in the art, for example by DNA

sequencing of the LD block A genomic region in particular group of individuals, and the present invention also encompasses such additional variants.

As described herein, certain markers within the exon 4 LD block of TCF7L2 are found in decreased frequency in individuals with type II diabetes, and haplotypes comprising two or more of those markers listed in Tables 13, 20 and 21 are also found to be present at decreased frequency in individuals with type II diabetes. These markers and haplotypes are thus protective for type II diabetes, i.e. they confer a decreased risk of individuals carrying these markers and/or haplotypes developing type II diabetes.

The haplotypes and markers described herein are, in some cases, a combination of various genetic markers, *e.g.*, SNPs and microsatellites. Therefore, detecting haplotypes can be accomplished by methods known in the art and/or described herein for detecting sequences at polymorphic sites. Furthermore, correlation between certain haplotypes or sets of markers and disease phenotype can be verified using standard techniques. A representative example of a simple test for correlation would be a Fisher-exact test on a two by two table.

In specific embodiments, a marker or haplotype associated with the exon 4 LD block of TCF7L2 is one in which the marker or haplotype is more frequently present in an individual at risk for type II diabetes (affected), compared to the frequency of its presence in a healthy individual (control), wherein the presence of the marker or haplotype is indicative of type II diabetes or a susceptibility to type II diabetes. In other embodiments, at-risk tagging markers in linkage disequilibrium with one or more markers associated with the exon 4 LD block of TCF7L2, are tagging markers that are more frequently present in an individual at risk for type II diabetes (affected), compared to the frequency of their presence in a healthy individual (control), wherein the presence of the tagging markers is indicative of increased susceptibility to type II diabetes. In a further embodiment, at-risk markers in linkage disequilibrium with one or more markers associated with the exon 4 LD block of TCF7L2, are markers that are more frequently present in an individual at risk for type II diabetes, compared to the frequency of their presence in a healthy individual (control), wherein the presence of the markers is indicative of susceptibility to type II diabetes.

In certain methods described herein, an individual who is at risk for type II diabetes is an individual in whom an at-risk marker or haplotype is identified,. In one

embodiment, the strength of the association of a marker or haplotype is measured by relative risk (RR). RR is the ratio of the incidence of the condition among subjects who carry one copy of the marker or haplotype to the incidence of the condition among subjects who do not carry the marker or haplotype. This ratio is equivalent to the ratio of the incidence of the condition among subjects who carry two copies of the marker or haplotype to the incidence of the condition among subjects who carry one copy of the marker or haplotype. In one embodiment, the marker or haplotype has a relative risk of at least 1.2. In other embodiments, the marker or haplotype has a relative risk of at least 1.3, at least 1.4, at least 1.5, at least 2.0, at least 2.5, at least 3.0, at least 3.5, at least 4.0, or at least 5.0.

In other methods of the invention, an individual who has a decreased risk (or deceased susceptibility) of type II diabetes is an individual in whom a protective marker or haplotype is identified. In such cases, the relative risk (RR) is less than unity. In one embodiment, the marker or haplotype has a relative risk of less than 0.9. In another embodiments, the marker or haplotype has a relative risk of less than 0.8, less than 0.7, less than 0.6, less than 0.5 or less than 0.4.

Utility of Genetic Testing

The knowledge about a genetic variant that confers a risk of developing type II diabetes offers the opportunity to apply a genetic-test to distinguish between individuals with increased risk of developing the disease (i.e. carriers of the at-risk variant) and those with decreased risk of developing the disease (i.e. carriers of the protective variant). The core values of genetic testing, for individuals belonging to both of the above mentioned groups, are the possibilities of being able to diagnose the disease at an early stage and provide information to the clinician about prognosis/aggressiveness of the disease in order to be able to apply the most appropriate treatment. For example, the application of a genetic test for type II diabetes can provide an opportunity for the detection of the disease at an earlier stage which may lead to the application of therapeutic measures at an earlier stage, and thus can minimize the deleterious effects of the symptoms and serious health consequences conferred by type II diabetes.

METHODS OF THERAPY

In another embodiment of the invention, methods can be employed for the treatment of type II diabetes. The term "treatment" as used herein, refers not only to ameliorating symptoms associated with type II diabetes, but also preventing or delaying the onset of type II diabetes; lessening the severity or frequency of symptoms of type II diabetes; and/or also lessening the need for concomitant therapy with other drugs that ameliorate symptoms associated with type II diabetes. In one aspect, the individual to be treated is an individual who is susceptible (at an increased risk) for type II diabetes (e.g., an individual having the presence of an allele other than a 0 allele in marker DG10S478; the presence of a T allele in SNP rs12255372; the presence of an A allele in SNP rs7895340; the presence of a C allele in SNP rs11196205; the presence of a C allele in SNP rs7901695; the presence of a T allele in SNP rs7903146; the presence of a C allele in SNP rs12243326; or the presence of an T allele in SNP rs4506565.

In additional embodiments of the invention, methods can be employed for the treatment of other diseases or conditions associated with *TCF7L2*. A TCF7L2 therapeutic agent can be used both in methods of treatment of type II diabetes, as well as in methods of treatment of other diseases or conditions associated with *TCF7L2*.

The methods of treatment (prophylactic and/or therapeutic) utilize a TCF7L2 therapeutic agent. A "TCF7L2 therapeutic agent" is an agent that alters (*e.g.*, enhances or inhibits) polypeptide activity and/or nucleic acid expression of *TCF7L2*, either directly or indirectly (*e.g.*, through altering activity or nucleic acid expression of a protein that interacts with *TCF7L2*, such as a protein in the Wnt signaling pathway or in the cadherin pathway (*e.g.*, beta-catenin)). In certain embodiments, the TCF7L2 therapeutic agent alters activity and/or nucleic acid expression of *TCF7L2*.

TCF7L2 therapeutic agents can alter TCF7L2 polypeptide activity or nucleic acid expression by a variety of means, such as, for example, by providing additional TCF7L2 polypeptide or by upregulating the transcription or translation of the *TCF7L2* nucleic acid; by altering posttranslational processing of the TCF7L2 polypeptide; by altering transcription of *TCF7L2* splicing variants; or by interfering with TCF7L2 polypeptide activity (*e.g.*, by binding to a TCF7L2 polypeptide), or by binding to another polypeptide that interacts with TCF7L2, by altering (*e.g.*, downregulating) the expression,

transcription or translation of a *TCF7L2* nucleic acid, or by altering (*e.g.*, agonizing or antagonizing) activity.

Representative TCF7L2 therapeutic agents include the following: nucleic acids or fragments or derivatives thereof described herein, particularly nucleotides encoding the polypeptides described herein and vectors comprising such nucleic acids (*e.g.*, a gene, cDNA, and/or mRNA, such as a nucleic acid encoding a TCF7L2 polypeptide or active fragment or derivative thereof, or an oligonucleotide; or a complement thereof, or fragments or derivatives thereof, and/or other splicing variants encoded by a Type II diabetes nucleic acid, or fragments or derivatives thereof); polypeptides described herein and/ or splicing variants encoded by the *TCF7L2* nucleic acid or fragments or derivatives thereof; other polypeptides (*e.g.*, TCF7L2 receptors); TCF7L2 binding agents; or agents that affect (*e.g.*, increase or decrease) activity, antibodies, such as an antibody to an altered TCF7L2 polypeptide, or an antibody to a non-altered TCF7L2 polypeptide, or an antibody to a particular splicing variant encoded by a *TCF7L2* nucleic acid as described above; peptidomimetics; fusion proteins or prodrugs thereof; ribozymes; other small molecules; and other agents that alter (*e.g.*, enhance or inhibit) expression of a *TCF7L2* nucleic acid, or that regulate transcription of *TCF7L2* splicing variants (*e.g.*, agents that affect which splicing variants are expressed, or that affect the amount of each splicing variant that is expressed). Additional representative *TCF7L2* therapeutic agents include compounds that influence insulin signaling and/or glucagons, GLP-1 or GIP signaling. More than one TCF7L2 therapeutic agent can be used concurrently, if desired.

In preferred embodiments, the TCF7L2 therapeutic agent is an agent that interferes with the activity of TCF7L2, such as, for example, an agent that interferes with TCF7L2 binding or interaction of TCF7L2 with beta-catenin (see, e.g., Fasolini, *et al.*, J. Biol. Chem 278(23):21092-06 (2003)) or with other proteins. Other TCF7L2 therapeutic agents include agents that affect the Wnt signaling pathway or agents that affect the cadherin pathway. Representative agents include agents such as those used for cancer therapy, including, for example, proteins such as the DKK proteins; the beta-catenin binding domain of APC, or Axin; factors such as IDAX, AXAM and ICAT; antisense oligonucleotides or RNA interference (RNAi), such as with the use of Vitravene; oncolytic viral vectors; and other compounds (see, e.g., Luu *et al.*, Current Cancer Drug Targets 4:6530671 (2004)); small molecule antagonists, including, for example,

ZTM00990, PKF118-310, PKF118-744, PKF115-584, PKF222-815, CGPO49090, NPDDG39.024, and NPDDG1.024 as described by Lepourcelet *et al.* (see, e.g., Lepourcelet *et al.*,, Cancer Call 5:91-102 (2004)); compounds described in US Patent 6,762,185; compounds described in US Patent applications 20040005313, 20040072831, 20040247593, or 20050059628. Other representative TCF7L2 therapeutic agents include gsk3 inhibitors, including, for example, those described in US Patents 6,057,117; 6,153,618; 6,417,185; 6,465,231; 6,489,344; 6,512,102; 6,608,063; 6,716,624; 6,800,632; and published US Patent applications 20030008866; 20030077798; 20030130289; 20030207883; 2000092535; and 200500851. The entire teachings of all of the references, patents and patent applications recited in the Specification are incorporated herein in their entirety.

Additional representative TCF7L2 therapeutic agents are shown in the Agent Table, below.

Agent table

Compound name(s)	**Compound name (generated using Autonom, ISIS Draw version 2.5 from MDL Information Systems)**	**Company**	**Compound Reference**	**Indications**
AR-0133418 (SN-4521)	1-(4-Methoxy-benzyl)-3-(5-nitro-thiazol-2-yl)-urea	AstraZeneca		AD
AR-025028	NSD	AstraZeneca		
CT-98023	N-[4-(2,4-Dichloro-phenyl)-5-(1H-imidazol-2-yl)-pyrimidin-2-yl]-N'-(5-nitro-pyridin-2-yl)-ethane-1,2-diamine	Chiron Corp		non-insulin dependent diabetes
CT-20026	NSD	Chiron Corp	Wagman et al., Curr Pharm. Des 2004: 10(10) 1105-37	non-insulin dependent diabetes
CT-21022	NSD	Chiron Corp		non-insulin dependent diabetes
CT-20014	NSD	Chiron Corp		non-insulin dependent diabetes
CT-21018	NSD	Chiron Corp		non-insulin dependent diabetes
CHIR-98025	NSD	Chiron Corp		non-insulin dependent diabetes
CHIR-99021	NSD	Chiron Corp	Wagman et al., Curr Pharm. Des 2004: 10(10) 1105-37	non-insulin dependent diabetes
CG-100179	NSD	CrystalGenomics and Yuyu	WO-2004065370	diabetes mellitus (Korea)
	4-[2-(4-Dimethylamino-3-nitro-phenylamino)-pyrimidin-4-yl]-	Cyclacel Ltd.		non-insulin dependent diabetes, among

Compound name(s)	Compound name (generated using Autonom, ISIS Draw version 2.5 from MDL Information Systems)	Company	Compound Reference	Indications
	3,5-dimethyl-1H-pyrrole-2-carbonitrile			others.
NP-01139, NP-031112, NP-03112, NP-00361	4-Benzyl-2-methyl-[1,2,4]thiadiazolidine-3,5-dione	Neuropharma SA		CNS disorders, AD
	3-[9-Fluoro-2-(piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl]-4-imidazo[1,2-a]pyridin-3-yl-pyrrole-2,5-dione	Eli Lilly & Co		non-insulin dependent diabetes
GW-784752x, GW-784775, SB-216763, SB-415286	Cyclopentanecarboxylic acid (6-pyridin-3-yl-furo[2,3-d]pyrimidin-4-yl)-amide	GSK	WO-03024447 (compound referenced: 4-[2-(2-bromophenyl)-4-(4-fluorophenyl)-1H-imidazol-5-yl]pyridine	non-insulin dependent diabetes, neurodegenerative disease
NNC-57-0511, NNC-57-0545, NNC-57-0588	1-(4-Amino-furazan-3-yl)-5-piperidin-1-ylmethyl-1H-[1,2,3]triazole-4-carboxylic acid [1-pyridin-4-yl-meth-(E)-ylidene]-hydrazide	Novo Nordisk		non-insulin dependent diabetes,
CP-70949	NSD	Pfizer		Hypoglycemic agent
VX-608	NSD			Cerebrovascular ischemia, non-insulin dependent diabetes
KP-403 class	NSD	Kinetek		Nuclear factor kappa B modulator, Anti-inflammatory, Cell cycle inhibitor, Glycogen synthase kinase-3 beta inhibitor
BYETTA (exenatide)	Exenatide: $C_{184}H_{282}N_{50}O_{60}S$ - Amino acid sequence:H-His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH_2	Amylin / Eli Lilly & Co		non-insulin dependent diabetes
Vildagliptin (LAF237)	NSD	Novartis		non-insulin dependent diabetes - DPP-4 inhibitor

NSD = No Structure disclosed (in Iddb3)

The TCF7L2 therapeutic agent(s) are administered in a therapeutically effective amount (*i.e.*, an amount that is sufficient for "treatment," as described above). The amount which will be therapeutically effective in the treatment of a particular individual's disorder or condition will depend on the symptoms and severity of the disease, and can be determined by standard clinical techniques. In addition, *in vitro* or *in vivo* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of a practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

In one embodiment, a nucleic acid (*e.g.*, a nucleic acid encoding a TCF7L2 polypeptide); or another nucleic acid that encodes a TCF7L2 polypeptide or a splicing variant, derivative or fragment thereof can be used, either alone or in a pharmaceutical composition as described above. For example, a *TCF7L2* gene or nucleic acid or a cDNA encoding a TCF7L2 polypeptide, either by itself or included within a vector, can be introduced into cells (either *in vitro* or *in vivo*) such that the cells produce native TCF7L2 polypeptide. If necessary, cells that have been transformed with the gene or cDNA or a vector comprising the gene, nucleic acid or cDNA can be introduced (or re-introduced) into an individual affected with the disease. Thus, cells which, in nature, lack native TCF7L2 expression and activity, or have altered *TCF7L2* expression and activity, or have expression of a disease-associated *TCF7L2* splicing variant, can be engineered to express the TCF7L2 polypeptide or an active fragment of the TCF7L2 polypeptide (or a different variant of the TCF7L2 polypeptide). In certain embodiments, nucleic acids encoding a TCF7L2 polypeptide, or an active fragment or derivative thereof, can be introduced into an expression vector, such as a viral vector, and the vector can be introduced into appropriate cells in an animal. Other gene transfer systems, including viral and nonviral transfer systems, can be used. Alternatively, nonviral gene transfer methods, such as calcium phosphate coprecipitation, mechanical techniques (*e.g.*, microinjection); membrane fusion-mediated transfer via liposomes; or direct DNA uptake, can also be used.

Alternatively, in another embodiment of the invention, a nucleic acid of the invention; a nucleic acid complementary to a nucleic acid of the invention; or a portion of

such a nucleic acid (*e.g.*, an oligonucleotide as described below), can be used in "antisense" therapy, in which a nucleic acid (*e.g.*, an oligonucleotide) which specifically hybridizes to the mRNA and/or genomic DNA of a Type II diabetes gene is administered or generated *in situ*. The antisense nucleic acid that specifically hybridizes to the mRNA and/or DNA inhibits expression of the TCF7L2 polypeptide, *e.g.*, by inhibiting translation and/or transcription. Binding of the antisense nucleic acid can be by conventional base pair complementarity, or, for example, in the case of binding to DNA duplexes, through specific interaction in the major groove of the double helix.

An antisense construct of the present invention can be delivered, for example, as an expression plasmid as described above. When the plasmid is transcribed in the cell, it produces RNA that is complementary to a portion of the mRNA and/or DNA which encodes the TCF7L2 polypeptide. Alternatively, the antisense construct can be an oligonucleotide probe that is generated *ex vivo* and introduced into cells; it then inhibits expression by hybridizing with the mRNA and/or genomic DNA of the polypeptide. In one embodiment, the oligonucleotide probes are modified oligonucleotides, which are resistant to endogenous nucleases, *e.g.*, exonucleases and/or endonucleases, thereby rendering them stable *in vivo*. Exemplary nucleic acid molecules for use as antisense oligonucleotides are phosphoramidate, phosphothioate and methylphosphonate analogs of DNA (see also U.S. Pat. Nos. 5,176,996; 5,264,564; and 5,256,775). Additionally, general approaches to constructing oligomers useful in antisense therapy are also described, for example, by Van der Krol *et al.*, (*BioTechniques* 6:958-976 (1988)); and Stein *et al.*, (*Cancer Res.* 48:2659-2668 (1988)). With respect to antisense DNA, oligodeoxyribonucleotides derived from the translation initiation site are preferred.

To perform antisense therapy, oligonucleotides (mRNA, cDNA or DNA) are designed that are complementary to mRNA encoding the TCF7L2 gene. The antisense oligonucleotides bind to *TCF7L2* mRNA transcripts and prevent translation. Absolute complementarity, although preferred, is not required. A sequence "complementary" to a portion of an RNA, as referred to herein, indicates that a sequence has sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid, as

described in detail above. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures.

The oligonucleotides used in antisense therapy can be DNA, RNA, or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotides can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotides can include other appended groups such as peptides (*e.g.* for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.*, *Proc. Natl. Acad. Sci. USA* 86:6553-6556 (1989); Lemaitre *et al.*, *Proc. Natl. Acad. Sci. USA* 84:648-652 (1987); PCT International Publication NO: WO 88/09810) or the blood-brain barrier (see, *e.g.*, PCT International Publication NO: WO 89/10134), or hybridization-triggered cleavage agents (see, *e.g.*, Krol *et al.*, *BioTechniques* 6:958-976 (1988)) or intercalating agents. (See, *e.g.*, Zon, Pharm. Res. 5:539-549 (1988)). To this end, the oligonucleotide may be conjugated to another molecule (*e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent).

The antisense molecules are delivered to cells that express *TCF7L2 in vivo*. A number of methods can be used for delivering antisense DNA or RNA to cells; *e.g.*, antisense molecules can be injected directly into the tissue site, or modified antisense molecules, designed to target the desired cells (*e.g.*, antisense linked to peptides or antibodies that specifically bind receptors or antigens expressed on the target cell surface) can be administered systematically. Alternatively, in a preferred embodiment, a recombinant DNA construct is utilized in which the antisense oligonucleotide is placed under the control of a strong promoter (*e.g.*, pol III or pol II). The use of such a construct to transfect target cells in the patient results in the transcription of sufficient amounts of single stranded RNAs that will form complementary base pairs with the endogenous TCF7L2 transcripts and thereby prevent translation of the TCF7L2 mRNA. For example, a vector can be introduced *in vivo* such that it is taken up by a cell and directs the transcription of an antisense RNA. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired

antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art and described above. For example, a plasmid, cosmid, YAC or viral vector can be used to prepare the recombinant DNA construct that can be introduced directly into the tissue site. Alternatively, viral vectors can be used which selectively infect the desired tissue, in which case administration may be accomplished by another route (*e.g.*, systemically).

Endogenous TCF7L2 polypeptide expression can also be reduced by inactivating or "knocking out" the gene, nucleic acid or its promoter using targeted homologous recombination (*e.g.*, see Smithies *et al.*, *Nature* 317:230-234 (1985); Thomas & Capecchi, *Cell* 51:503-512 (1987); Thompson *et al.*, *Cell* 5:313-321 (1989)). For example, an altered, non-functional gene or nucleic acid (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous gene or nucleic acid (either the coding regions or regulatory regions of the nucleic acid) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express the gene or nucleic acid *in vivo*. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the gene or nucleic acid. The recombinant DNA constructs can be directly administered or targeted to the required site *in vivo* using appropriate vectors, as described above. Alternatively, expression of non-altered genes or nucleic acids can be increased using a similar method: targeted homologous recombination can be used to insert a DNA construct comprising a non-altered functional gene or nucleic acid in place of an altered TCF7L2 in the cell, as described above. In another embodiment, targeted homologous recombination can be used to insert a DNA construct comprising a nucleic acid that encodes a Type II diabetes polypeptide variant that differs from that present in the cell.

Alternatively, endogenous *TCF7L2* nucleic acid expression can be reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of a *TCF7L2* nucleic acid (*i.e.*, the *TCF7L2* promoter and/or enhancers) to form triple helical structures that prevent transcription of the *TCF7L2* nucleic acid in target cells in the body. (See generally, Helene, C., *Anticancer Drug Des.*, 6(6):569-84 (1991); Helene, C. *et al.*, *Ann. N.Y. Acad. Sci.* 660:27-36 (1992); and Maher, L. J., *Bioassays* 14(12):807-15 (1992)). Likewise, the antisense constructs described herein, by antagonizing the normal biological activity of one of the TCF7L2 proteins, can be used in the manipulation of

tissue, *e.g.*, tissue differentiation, both *in vivo* and *for ex vivo* tissue cultures. Furthermore, the anti-sense techniques (*e.g.*, microinjection of antisense molecules, or transfection with plasmids whose transcripts are anti-sense with regard to a Type II diabetes gene mRNA or gene sequence) can be used to investigate the role of TCF7L2 or the interaction of TCF7L2 and its binding agents in developmental events, as well as the normal cellular function of TCF7L2 or of the interaction of TCF7L2 and its binding agents in adult tissue. Such techniques can be utilized in cell culture, but can also be used in the creation of transgenic animals.

In yet another embodiment of the invention, other TCF7L2 therapeutic agents as described herein can also be used in the treatment of Type II diabetes gene. The therapeutic agents can be delivered in a composition, as described above, or by themselves. They can be administered systemically, or can be targeted to a particular tissue. The therapeutic agents can be produced by a variety of means, including chemical synthesis; recombinant production; *in vivo* production (*e.g.*, a transgenic animal, such as U.S. Pat. NO: 4,873,316 to Meade *et al.*), for example, and can be isolated using standard means such as those described herein.

A combination of any of the above methods of treatment (*e.g.*, administration of non-altered polypeptide in conjunction with antisense therapy targeting altered mRNA of TCF7L2; administration of a first splicing variant encoded by a *TCF7L2* nucleic acid in conjunction with antisense therapy targeting a second splicing encoded by a *TCF7L2* nucleic acid) can also be used.

METHODS OF ASSESSING PROBABILITY OF RESPONSE TO TCF7L2 THERAPEUTIC AGENTS

The present invention additionally pertains to methods of assessing an individual's probability of response to a TCF7L2 therapeutic agent. In the methods, markers or haplotypes relating to the *TCF7L2* gene are assessed, as described above in relation to assessing an individual for susceptibility to type II diabetes. The presence of an allele, marker, SNP or haplotype associated with susceptibility (increased risk) for type II diabetes (e.g., an allele other than a 0 allele in marker DG10S478; a T allele in SNP rs12255372; an A allele in SNP rs7895340; a C allele in SNP rs11196205; a C allele in SNP rs7901695; a T allele in SNP rs7903146; a C allele in SNP rs12243326; an T allele

in SNP rs4506565; a marker associated with the exon 4 LD block of *TCF7L2*, such as an at-risk haplotype associated with the exon 4 LD block of *TCF7L2*); is indicative of a probability of a positive response to a TCF7L2 therapeutic agent. "Probability of a positive response" indicates that the individual is more likely to have a positive response to a TCF7L2 therapeutic agent than an individual not having an allele, marker, SNP or haplotype associated with susceptibility (increased risk) for type II diabetes as described herein. A "positive response" to a TCF7L2 therapeutic agent is a physiological response that indicates treatment of type II diabetes. As described above, "treatment" refers not only to ameliorating symptoms associated with type II diabetes, but also preventing or delaying the onset of type II diabetes; lessening the severity or frequency of symptoms of type II diabetes; and/or also lessening the need for concomitant therapy with other drugs that ameliorate symptoms associated with type II diabetes.

PHARMACEUTICAL COMPOSITIONS

The present invention also pertains to pharmaceutical compositions comprising agents that alter TCF7L2 activity or which otherwise affect the Wnt signaling pathway or the cadherin pathway, or which can be used as TCF7L2 therapeutic agents. The pharmaceutical compositions can be formulated with a physiologically acceptable carrier or excipient to prepare a pharmaceutical composition. The carrier and composition can be sterile. The formulation should suit the mode of administration.

Suitable pharmaceutically acceptable carriers include but are not limited to water, salt solutions (*e.g.*, NaCl), saline, buffered saline, alcohols, glycerol, ethanol, gum arabic, vegetable oils, benzyl alcohols, polyethylene glycols, gelatin, carbohydrates such as lactose, amylose or starch, dextrose, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid esters, hydroxymethylcellulose, polyvinyl pyrolidone, etc., as well as combinations thereof. The pharmaceutical preparations can, if desired, be mixed with auxiliary agents, *e.g.*, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or aromatic substances and the like which do not deleteriously react with the active agents.

The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. The

composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, polyvinyl pyrollidone, sodium saccharine, cellulose, magnesium carbonate, etc.

Methods of introduction of these compositions include, but are not limited to, intradermal, intramuscular, intraperitoneal, intraocular, intravenous, subcutaneous, topical, oral and intranasal. Other suitable methods of introduction can also include gene therapy (as described below), rechargeable or biodegradable devices, particle acceleration devises ("gene guns") and slow release polymeric devices. The pharmaceutical compositions of this invention can also be administered as part of a combinatorial therapy with other agents.

The composition can be formulated in accordance with the routine procedures as a pharmaceutical composition adapted for administration to human beings. For example, compositions for intravenous administration typically are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water, saline or dextrose/water. Where the composition is administered by injection, an ampule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

For topical application, nonsprayable forms, viscous to semi-solid or solid forms comprising a carrier compatible with topical application and having a dynamic viscosity preferably greater than water, can be employed. Suitable formulations include but are not limited to solutions, suspensions, emulsions, creams, ointments, powders, enemas, lotions, sols, liniments, salves, aerosols, etc., which are, if desired, sterilized or mixed with auxiliary agents, *e.g.*, preservatives, stabilizers, wetting agents, buffers or salts for influencing osmotic pressure, etc. The agent may be incorporated into a cosmetic formulation. For topical application, also suitable are sprayable aerosol preparations wherein the active ingredient, preferably in combination with a solid or liquid inert carrier

material, is packaged in a squeeze bottle or in admixture with a pressurized volatile, normally gaseous propellant, *e.g.*, pressurized air.

Agents described herein can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The agents are administered in a therapeutically effective amount. The amount of agents which will be therapeutically effective depends in part on the nature of the disorder and/or extent of symptoms, and can be determined by standard clinical techniques. In addition, *in vitro* or *in vivo* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the symptoms, and should be decided according to the judgment of a practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use of sale for human administration. The pack or kit can be labeled with information regarding mode of administration, sequence of drug administration (*e.g.*, separately, sequentially or concurrently), or the like. The pack or kit may also include means for reminding the patient to take the therapy. The pack or kit can be a single unit dosage of the combination therapy or it can be a plurality of unit dosages. In particular, the agents can be separated, mixed together in any combination, present in a single vial or tablet. Agents assembled in a blister pack or other dispensing means is preferred. For the purpose of this invention, unit dosage is intended to mean a dosage that is dependent on the individual pharmacodynamics of each agent and administered in FDA approved dosages in standard time courses.

SCREENING ASSAYS AND AGENTS IDENTIFIED THEREBY

The invention also provides methods for identifying agents (*e.g.*, fusion proteins, polypeptides, peptidomimetics, prodrugs, receptors, binding agents, antibodies, small molecules or other drugs, or ribozymes) which alter (*e.g.*, increase or decrease) the activity of the TCF7L2, which otherwise interact with TCF7L2 or with another member of the Wnt signaling pathway or the cadherin pathway (*e.g.*, beta-catenin). For example, in certain embodiments, such agents can be agents which bind to TCF7L2; which have a stimulatory or inhibitory effect on, for example, activity of TCF7L2; or which change (*e.g.*, enhance or inhibit) the ability of TCF7L2 to interact with other members of the Wnt signaling pathway or with members of the cadherin pathway, or which alter posttranslational processing of TCF7L2. In other embodiments, such agents can be agents which alter activity or function of the Wnt signaling pathway or the cadherin pathway.

In one embodiment, the invention provides assays for screening candidate or test agents that bind to or modulate the activity of TCF7L2 protein (or biologically active portion(s) thereof), as well as agents identifiable by the assays. Test agents can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide libraries, while the other four approaches are applicable to polypeptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S., *Anticancer Drug Des.* 12:145 (1997)).

In one embodiment, to identify agents which alter the activity of TCF7L2, a cell, cell lysate, or solution containing or expressing TCF7L2, or a fragment or derivative thereof, can be contacted with an agent to be tested; alternatively, the protein can be contacted directly with the agent to be tested. The level (amount) of TCF7L2 activity is assessed (*e.g.*, the level (amount) of TCF7L2 activity is measured, either directly or indirectly), and is compared with the level of activity in a control (*i.e.*, the level of activity of the TCF7L2 protein or active fragment or derivative thereof in the absence of the agent

to be tested). If the level of the activity in the presence of the agent differs, by an amount that is statistically significant, from the level of the activity in the absence of the agent, then the agent is an agent that alters the activity of TCF7L2. An increase in the level of activity relative to a control, indicates that the agent is an agent that enhances (is an agonist of) activity. Similarly, a decrease in the level of activity relative to a control, indicates that the agent is an agent that inhibits (is an antagonist of) activity. In another embodiment, the level of activity of TCF7L2 or a derivative or fragment thereof in the presence of the agent to be tested, is compared with a control level that has previously been established. A level of the activity in the presence of the agent that differs from the control level by an amount that is statistically significant indicates that the agent alters TCF7L2 activity.

The present invention also relates to an assay for identifying agents which alter the expression of the *TCF7L2* gene (*e.g.*, antisense nucleic acids, fusion proteins, polypeptides, peptidomimetics, prodrugs, receptors, binding agents, antibodies, small molecules or other drugs, or ribozymes) which alter (*e.g.*, increase or decrease) expression (*e.g.*, transcription or translation) of the gene or which otherwise interact with TCF7L2, as well as agents identifiable by the assays. For example, a solution containing a nucleic acid encoding a *TCF7L2* can be contacted with an agent to be tested. The solution can comprise, for example, cells containing the nucleic acid or cell lysate containing the nucleic acid; alternatively, the solution can be another solution that comprises elements necessary for transcription/translation of the nucleic acid. Cells not suspended in solution can also be employed, if desired. The level and/or pattern of TCF7L2 expression (*e.g.*, the level and/or pattern of mRNA or of protein expressed, such as the level and/or pattern of different splicing variants) is assessed, and is compared with the level and/or pattern of expression in a control (*i.e.*, the level and/or pattern of the TCF7L2 expression in the absence of the agent to be tested). If the level and/or pattern in the presence of the agent differs, by an amount or in a manner that is statistically significant, from the level and/or pattern in the absence of the agent, then the agent is an agent that alters the expression of a Type II diabetes gene. Enhancement of TCF7L2 expression indicates that the agent is an agonist of TCF7L2 activity. Similarly, inhibition of TCF7L2 expression indicates that the agent is an antagonist of TCF7L2 activity. In another embodiment, the level and/or pattern of TCF7L2 polypeptide(s) (*e.g.*, different

splicing variants) in the presence of the agent to be tested, is compared with a control level and/or pattern that have previously been established. A level and/or pattern in the presence of the agent that differs from the control level and/or pattern by an amount or in a manner that is statistically significant indicates that the agent alters TCF7L2 expression.

In another embodiment of the invention, agents which alter the expression of TCF7L2 or which otherwise interact with TCF7L2 or with another member of the Wnt signaling pathway or the cadherin pathway, can be identified using a cell, cell lysate, or solution containing a nucleic acid encoding the promoter region of the *TCF7L2* gene or nucleic acid operably linked to a reporter gene. After contact with an agent to be tested, the level of expression of the reporter gene (*e.g.*, the level of mRNA or of protein expressed) is assessed, and is compared with the level of expression in a control (*i.e.*, the level of the expression of the reporter gene in the absence of the agent to be tested). If the level in the presence of the agent differs, by an amount or in a manner that is statistically significant, from the level in the absence of the agent, then the agent is an agent that alters the expression of TCF7L2, as indicated by its ability to alter expression of a gene that is operably linked to the *TCF7L2* gene promoter. Enhancement of the expression of the reporter indicates that the agent is an agonist of TCF7L2 activity. Similarly, inhibition of the expression of the reporter indicates that the agent is an antagonist of TCF7L2 activity. In another embodiment, the level of expression of the reporter in the presence of the agent to be tested is compared with a control level that has previously been established. A level in the presence of the agent that differs from the control level by an amount or in a manner that is statistically significant indicates that the agent alters expression.

Agents which alter the amounts of different splicing variants encoded by *TCF7L2* (*e.g.*, an agent which enhances activity of a first splicing variant, and which inhibits activity of a second splicing variant), as well as agents which are agonists of activity of a first splicing variant and antagonists of activity of a second splicing variant, can easily be identified using these methods described above.

In other embodiments of the invention, assays can be used to assess the impact of a test agent on the activity of a polypeptide in relation to a TCF7L2 binding agent. For example, a cell that expresses a compound that interacts with a TCF7L2 polypeptide (herein referred to as a " TCF7L2 binding agent", which can be a polypeptide or other molecule that interacts directly or indirectly with a TCF7L2 polypeptide, such as a

member of the Wnt signaling pathway or a member of the cadherin pathway) is contacted with TCF7L2 in the presence of a test agent, and the ability of the test agent to alter the interaction between the TCF7L2 and the TCF7L2 binding agent is determined. Alternatively, a cell lysate or a solution containing the TCF7L2 binding agent, can be used. An agent that binds to the TCF7L2 or the TCF7L2 binding agent can alter the interaction by interfering with, or enhancing the ability of the TCF7L2 to bind to, associate with, or otherwise interact with the TCF7L2 binding agent. Determining the ability of the test agent to bind to TCF7L2 or a TCF7L2 binding agent can be accomplished, for example, by coupling the test agent with a radioisotope or enzymatic label such that binding of the test agent to the polypeptide can be determined by detecting the labeled with ^{125}I, ^{35}S, ^{14}C or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, test agents can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. It is also within the scope of this invention to determine the ability of a test agent to interact with the polypeptide without the labeling of any of the interactants. For example, a microphysiometer can be used to detect the interaction of a test agent with TCF7L2 or a TCF7L2 binding agent without the labeling of either the test agent, TCF7L2, or the TCF7L2 binding agent. McConnell, H.M. *et al.*, *Science* 257:1906-1912 (1992). As used herein, a "microphysiometer" (*e.g.*, Cytosensor™) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between ligand and polypeptide.

Thus, these receptors can be used to screen for compounds that are agonists or antagonists, for use in treating or studying a susceptibility to type II diabetes. Drugs could be designed to regulate TCF7L2 activation that in turn can be used to regulate signaling pathways and transcription events of genes downstream.

In another embodiment of the invention, assays can be used to identify polypeptides that interact with TCF7L2. For example, a yeast two-hybrid system such as that described by Fields and Song (Fields, S. and Song, O., *Nature* 340:245-246 (1989)) can be used to identify polypeptides that interact with TCF7L2. In such a yeast two-

hybrid system, vectors are constructed based on the flexibility of a transcription factor that has two functional domains (a DNA binding domain and a transcription activation domain). If the two domains are separated but fused to two different proteins that interact with one another, transcriptional activation can be achieved, and transcription of specific markers (*e.g.*, nutritional markers such as His and Ade, or color markers such as lacZ) can be used to identify the presence of interaction and transcriptional activation. For example, in the methods of the invention, a first vector is used which includes a nucleic acid encoding a DNA binding domain and also *TCF7L2*, splicing variant, or fragment or derivative thereof, and a second vector is used which includes a nucleic acid encoding a transcription activation domain and also a nucleic acid encoding a polypeptide which potentially may interact with TCF7L2 or a splicing variant, or fragment or derivative thereof. Incubation of yeast containing the first vector and the second vector under appropriate conditions (*e.g.*, mating conditions such as used in the Matchmaker™ system from Clontech (Palo Alto, California, USA)) allows identification of colonies that express the markers of interest. These colonies can be examined to identify the polypeptide(s) that interact with TCF7L2 or fragment or derivative thereof. Such polypeptides can be used as agents that alter the activity of expression of TCF7L2, as described in relation to methods of treatment.

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either the *TCF7L2* gene, the TCF7L2 protein, the TCF7L2 binding agent (e.g., another member of the Wnt signaling pathway or member of the cadherin pathway), or other components of the assay on a solid support, in order to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test agent to the protein, or interaction of the protein with a binding agent in the presence and absence of a test agent, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein (*e.g.*, a glutathione-S-transferase fusion protein) can be provided which adds a domain that allows TCF7L2, TCF7L2 protein, or a TCF7L2 binding agent to be bound to a matrix or other solid support.

In another embodiment, modulators of expression of nucleic acid molecules of the invention are identified in a method wherein a cell, cell lysate, or solution containing

TCF7L2 is contacted with a test agent and the expression of appropriate mRNA or polypeptide (*e.g.*, splicing variant(s)) in the cell, cell lysate, or solution, is determined. The level of expression of appropriate mRNA or polypeptide(s) in the presence of the test agent is compared to the level of expression of mRNA or polypeptide(s) in the absence of the test agent. The test agent can then be identified as a modulator of expression based on this comparison. For example, when expression of mRNA or polypeptide is greater (statistically significantly greater) in the presence of the test agent than in its absence, the test agent is identified as a stimulator or enhancer of the mRNA or polypeptide expression. Alternatively, when expression of the mRNA or polypeptide is less (statistically significantly less) in the presence of the test agent than in its absence, the test agent is identified as an inhibitor of the mRNA or polypeptide expression. The level of mRNA or polypeptide expression in the cells can be determined by methods described herein for detecting mRNA or polypeptide.

This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein in the methods of treatment described herein. For example, an agent identified as described herein can be used to alter activity of a protein encoded by a *TCF7L2* gene, or to alter expression of TCF7L2 by contacting the protein or the nucleic acid (or contacting a cell comprising the polypeptide or the nucleic acid) with the agent identified as described herein.

NUCLEIC ACIDS OF THE INVENTION

TCF7L2 Nucleic Acids, Portions and Variants

The present invention also pertains to isolated nucleic acid molecules comprising human *TCF7L2*. The *TCF7L2* nucleic acid molecules of the present invention can be RNA, for example, mRNA, or DNA, such as cDNA and genomic DNA. DNA molecules can be double-stranded or single-stranded; single stranded RNA or DNA can be the coding, or sense, strand or the non-coding, or antisense strand. The nucleic acid molecule can include all or a portion of the coding sequence of the gene and can further comprise additional non-coding sequences such as introns and non-coding 3' and 5' sequences (including regulatory sequences, for example).

Additionally, nucleic acid molecules of the invention can be fused to a marker sequence, for example, a sequence that encodes a polypeptide to assist in isolation or purification of the polypeptide. Such sequences include, but are not limited to, those that encode a glutathione-S-transferase (GST) fusion protein and those that encode a hemagglutinin A (HA) polypeptide marker from influenza.

An "isolated" nucleic acid molecule, as used herein, is one that is separated from nucleic acids that normally flank the gene or nucleotide sequence (as in genomic sequences) and/or has been completely or partially purified from other transcribed sequences (*e.g.*, as in an RNA library). For example, an isolated nucleic acid of the invention may be substantially isolated with respect to the complex cellular milieu in which it naturally occurs, or culture medium when produced by recombinant techniques, or chemical precursors or other chemicals when chemically synthesized. In some instances, the isolated material will form part of a composition (for example, a crude extract containing other substances), buffer system or reagent mix. In other circumstances, the material may be purified to essential homogeneity, for example as determined by PAGE or column chromatography such as HPLC. Preferably, an isolated nucleic acid molecule comprises at least about 50, 80 or 90% (on a molar basis) of all macromolecular species present. With regard to genomic DNA, the term "isolated" also can refer to nucleic acid molecules that are separated from the chromosome with which the genomic DNA is naturally associated. For example, the isolated nucleic acid molecule can contain less than about 5 kb but not limited to 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotides which flank the nucleic acid molecule in the genomic DNA of the cell from which the nucleic acid molecule is derived.

The nucleic acid molecule can be fused to other coding or regulatory sequences and still be considered isolated. Thus, recombinant DNA contained in a vector is included in the definition of "isolated" as used herein. Also, isolated nucleic acid molecules include recombinant DNA molecules in heterologous host cells, as well as partially or substantially purified DNA molecules in solution. "Isolated" nucleic acid molecules also encompass *in vivo* and *in vitro* RNA transcripts of the DNA molecules of the present invention. An isolated nucleic acid molecule can include a nucleic acid molecule or nucleic acid sequence that is synthesized chemically or by recombinant means. Therefore, recombinant DNA contained in a vector is included in the definition of

"isolated" as used herein. Also, isolated nucleic acid molecules include recombinant DNA molecules in heterologous organisms, as well as partially or substantially purified DNA molecules in solution. *In vivo* and *in vitro* RNA transcripts of the DNA molecules of the present invention are also encompassed by "isolated" nucleic acid sequences. Such isolated nucleic acid molecules are useful in the manufacture of the encoded polypeptide, as probes for isolating homologous sequences (*e.g.*, from other mammalian species), for gene mapping (*e.g.*, by *in situ* hybridization with chromosomes), or for detecting expression of the gene in tissue (*e.g.*, human tissue), such as by Northern or Southern blot analysis.

The present invention also pertains to nucleic acid molecules which are not necessarily found in nature but which encode a TCF7L2 polypeptide, or another splicing variant of a TCF7L2 polypeptide or polymorphic variant thereof. Thus, for example, the invention pertains to DNA molecules comprising a sequence that is different from the naturally occurring nucleotide sequence but which, due to the degeneracy of the genetic code, encode a TCF7L2 polypeptide of the present invention. The invention also encompasses nucleic acid molecules encoding portions (fragments), or encoding variant polypeptides such as analogues or derivatives of a TCF7L2 polypeptide. Such variants can be naturally occurring, such as in the case of allelic variation or single nucleotide polymorphisms, or non-naturally-occurring, such as those induced by various mutagens and mutagenic processes. Intended variations include, but are not limited to, addition, deletion and substitution of one or more nucleotides that can result in conservative or non-conservative amino acid changes, including additions and deletions. Preferably the nucleotide (and/or resultant amino acid) changes are silent or conserved; that is, they do not alter the characteristics or activity of a TCF7L2 polypeptide. In one aspect, the nucleic acid sequences are fragments that comprise one or more polymorphic microsatellite markers. In another aspect, the nucleotide sequences are fragments that comprise one or more single nucleotide polymorphisms in a *TCF7L2* gene.

Other alterations of the nucleic acid molecules of the invention can include, for example, labeling, methylation, internucleotide modifications such as uncharged linkages (*e.g.*, methyl phosphonates, phosphotriesters, phosphoamidates, carbamates), charged linkages (*e.g.*, phosphorothioates, phosphorodithioates), pendent moieties (*e.g.*, polypeptides), intercalators (*e.g.*, acridine, psoralen), chelators, alkylators, and modified

linkages (*e.g.*, alpha anomeric nucleic acids). Also included are synthetic molecules that mimic nucleic acid molecules in the ability to bind to a designated sequence via hydrogen bonding and other chemical interactions. Such molecules include, for example, those in which peptide linkages substitute for phosphate linkages in the backbone of the molecule.

The invention also pertains to nucleic acid molecules that hybridize under high stringency hybridization conditions, such as for selective hybridization, to a nucleotide sequence described herein (*e.g.*, nucleic acid molecules which specifically hybridize to a nucleotide sequence encoding polypeptides described herein, and, optionally, have an activity of the polypeptide). In one aspect, the invention includes variants described herein that hybridize under high stringency hybridization conditions (*e.g.*, for selective hybridization) to a nucleotide sequence encoding an amino acid sequence or a polymorphic variant thereof. In another aspect, the variant that hybridizes under high stringency hybridizations has an activity of a TCF7L2 polypeptide.

Such nucleic acid molecules can be detected and/or isolated by specific hybridization (*e.g.*, under high stringency conditions). "Specific hybridization," as used herein, refers to the ability of a first nucleic acid to hybridize to a second nucleic acid in a manner such that the first nucleic acid does not hybridize to any nucleic acid other than to the second nucleic acid (*e.g.*, when the first nucleic acid has a higher similarity to the second nucleic acid than to any other nucleic acid in a sample wherein the hybridization is to be performed). "Stringency conditions" for hybridization is a term of art which refers to the incubation and wash conditions, *e.g.*, conditions of temperature and buffer concentration, which permit hybridization of a particular nucleic acid to a second nucleic acid; the first nucleic acid may be perfectly (*i.e.*, 100%) complementary to the second, or the first and second may share some degree of complementarity which is less than perfect (*e.g.*, 70%, 75%, 85%, 90%, 95%). For example, certain high stringency conditions can be used which distinguish perfectly complementary nucleic acids from those of less complementarity. "High stringency conditions", "moderate stringency conditions" and "low stringency conditions", as well as methods for nucleic acid hybridizations are explained on pages 2.10.1-2.10.16 and pages 6.3.1-6.3.6 in *Current Protocols in Molecular Biology* (Ausubel, F. *et al.*, "*Current Protocols in Molecular Biology*", John Wiley & Sons, (1998)), and in Kraus, M. and Aaronson, S., *Methods Enzymol.*, 200:546-556 (1991),

The percent homology or identity of two nucleotide or amino acid sequences can be determined by aligning the sequences for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first sequence for optimal alignment). The nucleotides or amino acids at corresponding positions are then compared, and the percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % identity = # of identical positions/total # of positions x 100). When a position in one sequence is occupied by the same nucleotide or amino acid residue as the corresponding position in the other sequence, then the molecules are homologous at that position. As used herein, nucleic acid or amino acid "homology" is equivalent to nucleic acid or amino acid "identity". In certain aspects, the length of a sequence aligned for comparison purposes is at least 30%, for example, at least 40%, in certain aspects at least 60%, and in other aspects at least 70%, 80%, 90% or 95% of the length of the reference sequence. The actual comparison of the two sequences can be accomplished by well-known methods, for example, using a mathematical algorithm. A preferred, non-limiting example of such a mathematical algorithm is described in Karlin *et al.*, *Proc. Natl. Acad. Sci. USA* 90:5873-5877 (1993). Such an algorithm is incorporated into the NBLAST and XBLAST programs (version 2.0) as described in Altschul *et al.*, *Nucleic Acids Res.* 25:389-3402 (1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, NBLAST) can be used. In one aspect, parameters for sequence comparison can be set at score=100, wordlength=12, or can be varied (*e.g.*, W=5 or W=20).

Another preferred non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, *CABIOS* 4(1): 11-17 (1988). Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package (Accelrys, Cambridge, UK). When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used. Additional algorithms for sequence analysis are known in the art and include ADVANCE and ADAM as described in Torellis and Robotti, *Comput. Appl. Biosci.* 10:3-5 (1994); and FASTA described in Pearson and Lipman, *Proc. Natl. Acad. Sci. USA* 85:2444-8 (1988).

In another aspect, the percent identity between two amino acid sequences can be accomplished using the GAP program in the GCG software package using either a BLOSUM63 matrix or a PAM250 matrix, and a gap weight of 12, 10, 8, 6, or 4 and a length weight of 2, 3, or 4. In yet another aspect, the percent identity between two nucleic acid sequences can be accomplished using the GAP program in the GCG software package using a gap weight of 50 and a length weight of 3.

The present invention also provides isolated nucleic acid molecules that contain a fragment or portion that hybridizes under highly stringent conditions to a nucleotide sequence of *TCF7L2*, or the complement of such a sequence, and also provides isolated nucleic acid molecules that contain a fragment or portion that hybridizes under highly stringent conditions to a nucleotide sequence encoding an amino acid sequence or polymorphic variant thereof. The nucleic acid fragments of the invention are at least about 15, preferably at least about 18, 20, 23 or 25 nucleotides, and can be 30, 40, 50, 100, 200 or more nucleotides in length. Longer fragments, for example, 30 or more nucleotides in length, which encode antigenic polypeptides described herein, are particularly useful, such as for the generation of antibodies as described below.

Probes and Primers

In a related aspect, the nucleic acid fragments of the invention are used as probes or primers in assays such as those described herein. "Probes" or "primers" are oligonucleotides that hybridize in a base-specific manner to a complementary strand of nucleic acid molecules. Such probes and primers include polypeptide nucleic acids, as described in Nielsen *et al.*, *Science* 254:1497-1500 (1991).

A probe or primer comprises a region of nucleotide sequence that hybridizes to at least about 15, for example about 20-25, and in certain aspects about 40, 50 or 75, consecutive nucleotides of a nucleic acid molecule comprising a contiguous nucleotide sequence of *TCF7L2* or polymorphic variant thereof. In other aspects, a probe or primer comprises 100 or fewer nucleotides, in certain aspects from 6 to 50 nucleotides, for example from 12 to 30 nucleotides. In other aspects, the probe or primer is at least 70% identical to the contiguous nucleotide sequence or to the complement of the contiguous nucleotide sequence, for example at least 80% identical, in certain aspects at least 90% identical, and in other aspects at least 95% identical, or even capable of selectively hybridizing to the contiguous nucleotide sequence or to the complement of the contiguous

nucleotide sequence. Often, the probe or primer further comprises a label, *e.g.*, radioisotope, fluorescent compound, enzyme, or enzyme co-factor.

The nucleic acid molecules of the invention such as those described above can be identified and isolated using standard molecular biology techniques and the sequence information provided herein. For example, nucleic acid molecules can be amplified and isolated by the polymerase chain reaction using synthetic oligonucleotide primers designed based on the sequence of *TCF7L2* or the complement of such a sequence, or designed based on nucleotides based on sequences encoding one or more of the amino acid sequences provided herein. See generally *PCR Technology: Principles and Applications for DNA Amplification* (ed. H.A. Erlich, Freeman Press, NY, NY, 1992); *PCR Protocols: A Guide to Methods and Applications* (Eds. Innis *et al.*, Academic Press, San Diego, CA, 1990); Mattila *et al.*, *Nucl. Acids Res.* 19: 4967 (1991); Eckert *et al.*, *PCR Methods and Applications* 1:17 (1991); PCR (eds. McPherson *et al.*, IRL Press, Oxford); and U.S. Patent 4,683,202. The nucleic acid molecules can be amplified using cDNA, mRNA or genomic DNA as a template, cloned into an appropriate vector and characterized by DNA sequence analysis.

Other suitable amplification methods include the ligase chain reaction (LCR) (see Wu and Wallace, *Genomics* 4:560 (1989), Landegren *et al.*, *Science* 241:1077 (1988), transcription amplification (Kwoh *et al.*, *Proc. Natl. Acad. Sci. USA* 86:1173 (1989)), and self-sustained sequence replication (Guatelli *et al.*, *Proc. Nat. Acad. Sci. USA* 87:1874 (1990)) and nucleic acid based sequence amplification (NASBA). The latter two amplification methods involve isothermal reactions based on isothermal transcription, which produce both single stranded RNA (ssRNA) and double stranded DNA (dsDNA) as the amplification products in a ratio of about 30 or 100 to 1, respectively.

The amplified DNA can be labeled, for example, radiolabeled, and used as a probe for screening a cDNA library derived from human cells, mRNA in zap express, ZIPLOX or other suitable vector. Corresponding clones can be isolated, DNA can obtained following *in vivo* excision, and the cloned insert can be sequenced in either or both orientations by art recognized methods to identify the correct reading frame encoding a polypeptide of the appropriate molecular weight. For example, the direct analysis of the nucleotide sequence of nucleic acid molecules of the present invention can be accomplished using well-known methods that are commercially available. See, for

example, Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual* (2nd Ed., CSHP, New York 1989); Zyskind *et al.*, *Recombinant DNA Laboratory Manual*, (Acad. Press, 1988)). Additionally, fluorescence methods are also available for analyzing nucleic acids (Chen *et al., Genome Res.* 9, 492 (1999)) and polypeptides. Using these or similar methods, the polypeptide and the DNA encoding the polypeptide can be isolated, sequenced and further characterized.

Antisense nucleic acid molecules of the invention can be designed using the nucleotide sequence of *TCF7L2* and/or the complement or a portion, and constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid molecule (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used. Alternatively, the antisense nucleic acid molecule can be produced biologically using an expression vector into which a nucleic acid molecule has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid molecule will be of an antisense orientation to a target nucleic acid of interest).

The nucleic acid sequences can also be used to compare with endogenous DNA sequences in patients to identify one or more of the disorders described above, and as probes, such as to hybridize and discover related DNA sequences or to subtract out known sequences from a sample. The nucleic acid sequences can further be used to derive primers for genetic fingerprinting, to raise anti-polypeptide antibodies using DNA immunization techniques, and as an antigen to raise anti-DNA antibodies or elicit immune responses. Portions or fragments of the nucleotide sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways, such as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. Additionally, the nucleotide sequences of the invention can be used to identify and express recombinant polypeptides for analysis, characterization or therapeutic use, or as markers for tissues in which the

corresponding polypeptide is expressed, either constitutively, during tissue differentiation, or in diseased states. The nucleic acid sequences can additionally be used as reagents in the screening and/or diagnostic assays described herein, and can also be included as components of kits (*e.g.*, reagent kits) for use in the screening and/or diagnostic assays described herein.

Kits (*e.g.*, reagent kits) useful in the methods of diagnosis comprise components useful in any of the methods described herein, including for example, hybridization probes or primers as described herein (*e.g.*, labeled probes or primers), reagents for detection of labeled molecules, restriction enzymes (*e.g.*, for RFLP analysis), allele-specific oligonucleotides, antibodies which bind to altered or to non-altered (native) TCF7L2 polypeptide, means for amplification of nucleic acids comprising a *TCF7L2* nucleic acid or for a portion of *TCF7L2*, or means for analyzing the nucleic acid sequence of a *TCF7L2* nucleic acid or for analyzing the amino acid sequence of a TCF7L2 polypeptide as described herein, etc. In one aspect, the kit for diagnosing a susceptibility to type II diabetes can comprise primers for nucleic acid amplification of a region in the *TCF7L2* nucleic acid comprising the marker DG10S478, the SNP rs12255372, rs895340, rs11196205, rs7901695, rs7903146, rs12243326 and/or rs4506565, or an at-risk haplotype that is more frequently present in an individual having type II diabetes or who is susceptible to type II diabetes. The primers can be designed using portions of the nucleic acids flanking SNPs that are indicative of type II diabetes.

VECTORS AND HOST CELLS

Another aspect of the invention pertains to nucleic acid constructs containing a nucleic acid molecules described herein and the complements thereof (or a portion thereof). The constructs comprise a vector (*e.g.*, an expression vector) into which a sequence of the invention has been inserted in a sense or antisense orientation. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin

of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Expression vectors are capable of directing the expression of genes to which they are operably linked. In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses) that serve equivalent functions.

In certain aspects, recombinant expression vectors of the invention comprise a nucleic acid molecule of the invention in a form suitable for expression of the nucleic acid molecule in a host cell. This means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operably linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" or "operatively linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel, "Gene Expression Technology", *Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cell and those which direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed and the level of expression of polypeptide desired. The expression vectors of the invention can be introduced into host cells to thereby produce polypeptides, including fusion polypeptides, encoded by nucleic acid molecules as described herein.

The recombinant expression vectors of the invention can be designed for expression of a polypeptide of the invention in prokaryotic or eukaryotic cells, *e.g.*, bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors), yeast

cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *supra*. Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, a nucleic acid molecule of the invention can be expressed in bacterial cells (*e.g.*, *E. coli*), insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing a foreign nucleic acid molecule (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.*, (*supra*), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, for resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid molecules encoding a selectable marker can be introduced into a host cell on the same vector as the nucleic acid molecule of the invention or can be introduced on a separate vector. Cells stably transfected with the

introduced nucleic acid molecule can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture can be used to produce (*i.e.*, express) a polypeptide of the invention. Accordingly, the invention further provides methods for producing a polypeptide using the host cells of the invention. In one aspect, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding a polypeptide of the invention has been introduced) in a suitable medium such that the polypeptide is produced. In another aspect, the method further comprises isolating the polypeptide from the medium or the host cell.

ANTIBODIES OF THE INVENTION

Polyclonal antibodies and/or monoclonal antibodies that specifically bind one form of the gene product but not to the other form of the gene product are also provided. Antibodies are also provided which bind a portion of either the variant or the reference gene product that contains the polymorphic site or sites. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain antigen-binding sites that specifically bind an antigen. A molecule that specifically binds to a polypeptide of the invention is a molecule that binds to that polypeptide or a fragment thereof, but does not substantially bind other molecules in a sample, *e.g.*, a biological sample, which naturally contains the polypeptide. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and $F(ab')_2$ fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind to a polypeptide of the invention. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of a polypeptide of the invention. A monoclonal antibody composition thus typically displays a single binding affinity for a particular polypeptide of the invention with which it immunoreacts.

Polyclonal antibodies can be prepared as described above by immunizing a suitable subject with a desired immunogen, *e.g.*, polypeptide of the invention or a

fragment thereof. The antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized polypeptide. If desired, the antibody molecules directed against the polypeptide can be isolated from the mammal (*e.g.*, from the blood) and further purified by well-known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, *e.g.*, when the antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein, *Nature* 256:495-497 (1975), the human B cell hybridoma technique (Kozbor *et al.*, *Immunol. Today* 4: 72 (1983)), the EBV-hybridoma technique (Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss,1985, Inc., pp. 77-96) or trioma techniques. The technology for producing hybridomas is well known (see generally *Current Protocols in Immunology* (1994) Coligan *et al.*, (eds.) John Wiley & Sons, Inc., New York, NY). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with an immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds a polypeptide of the invention.

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating a monoclonal antibody to a polypeptide of the invention (see, *e.g.*, *Current Protocols in Immunology, supra*; Galfre *et al.*, *Nature* 266:55052 (1977); R.H. Kenneth, in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); and Lerner, *Yale J. Biol. Med.* 54:387-402 (1981)). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods that also would be useful.

Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal antibody to a polypeptide of the invention can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (*e.g.*, an antibody phage display library) with the polypeptide to thereby isolate immunoglobulin library members that bind the polypeptide. Kits for generating and screening phage display libraries are commercially available (*e.g.*, the Pharmacia *Recombinant Phage Antibody System*,

Catalog No. 27-9400-01; and the Stratagene *SurfZAP*™ Phage Display Kit, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, U.S. Patent No. 5,223,409; PCT Publication No. WO 92/18619; PCT Publication No. WO 91/17271; PCT Publication No. WO 92/20791; PCT Publication No. WO 92/15679; PCT Publication No. WO 93/01288; PCT Publication No. WO 92/01047; PCT Publication No. WO 92/09690; PCT Publication No. WO 90/02809; Fuchs *et al.*, *Bio/Technology* 9: 1370-1372 (1991); Hay *et al.*, *Hum. Antibod. Hybridomas* 3:81-85 (1992); Huse *et al.*, *Science* 246: 1275-1281 (1989); and Griffiths *et al.*, *EMBO J.* 12:725-734 (1993).

Additionally, recombinant antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art.

In general, antibodies of the invention (*e.g.*, a monoclonal antibody) can be used to isolate a polypeptide of the invention by standard techniques, such as affinity chromatography or immunoprecipitation. A polypeptide-specific antibody can facilitate the purification of natural polypeptide from cells and of recombinantly produced polypeptide expressed in host cells. Moreover, an antibody specific for a polypeptide of the invention can be used to detect the polypeptide (*e.g.*, in a cellular lysate, cell supernatant, or tissue sample) in order to evaluate the abundance and pattern of expression of the polypeptide. Antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. The antibody can be coupled to a detectable substance to facilitate its detection. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of

bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

The present invention is now illustrated by the following Exemplification, which is not intended to be limiting in any way.

EXEMPLIFICATION

Described herein is the identification of transcription factor 7-like 2 (*TCF7L2* - formerly *TCF4*) as a gene conferring risk of type II diabetes through single-point association analysis using a dense set of microsatellite markers within the 10q locus.

METHODS

Icelandic cohort

The Data Protection Authority of Iceland and the National Bioethics Committee of Iceland approved the study. All participants in the study gave informed consent. All personal identifiers associated with blood samples, medical information, and genealogy were first encrypted by the Data Protection Authority, using a third-party encryption system (*18*).

For this study, 2400 type II diabetes patients were identified who were diagnosed either through a long-term epidemiologic study done at the Icelandic Heart Association over the past 30 years or at one of two major hospitals in Reykjavik over the past 12 years. Two-thirds of these patients were alive, representing about half of the population of known type II diabetes patients in Iceland today. The majority of these patients were contacted for this study, and the cooperation rate exceeded 80%. All participants in the study visited the Icelandic Heart Association where they answered a questionnaire, had blood drawn and a fasting plasma glucose measurements taken. Questions about medication and age at diagnosis were included. The type II diabetes patients in this study were diagnosed as described in our previously published linkage study(*10*). In brief, the diagnosis of type II diabetes was confirmed by study physicians through previous medical records, medication history, and/or new laboratory measurements. For previously diagnosed type II diabetes patients, reporting of the use of oral glucose-lowering agent confirmed type II diabetes. Individuals who were currently treated with insulin were classified as having type II diabetes if they were also using or had previously used oral glucose-lowering agents. In this cohort the majority of patients on medication take oral glucose-lowering agents and only a small portion (9%)

require insulin. For hitherto undiagnosed individuals, the diagnosis of type II diabetes and impaired fasting glucose (IFG) was based on the criteria set by the American Diabetes Association (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 1997). The average age of the type II diabetes patients in this study was 69.7 years.

Replication cohorts

The Danish study group was selected from the PERF (Prospective Epidemiological Risk Factors) study in Denmark(*19*). 228 females had been diagnosed previously with type II diabetes and/or measured >=7mM glucose. As controls, 539 unaffected (with respect to type II diabetes) females were randomly drawn from the same study cohort.

The PENN CATH study in the US is a cross sectional study of the association of biochemical and genetic factors with coronary atherosclerosis in a consecutive cohort of patients undergoing cardiac catheterization at the University of Pennsylvania Medical Center between July 1998 and March 2003. Type II diabetes was defined as history of fasting blood glucose ≥126mg/dl, 2-hour post-prandial glucose ≥200mg/dl, use of oral hypoglycemic agents, or insulin and oral hypoglycemic in a subject greater than age 40. The University of Pennsylvania Institutional Review Board approved the study protocol and all subjects gave written informed consent. Ethnicity was determined through self-report. 361 Caucasian type II diabetes cases were derived from this cohort. 530 unaffected (with respect to type II diabetes and myocardial infarction) Caucasian controls were randomly drawn from the same study.

The DNA used for genotyping was the product of whole-genome amplification, by use of the GenomiPhi Amplification kit (Amersham), of DNA isolated from the peripheral blood of the Danish and US type II diabetes patients and controls.

Genotyping

New sequence repeats (i.e. dinucleotide, trinucleotide, and tetronucleotide repeats) were identified using the Tandem repeats finder software(*20*) and tested for polymorphicity in 94 controls. The size in basepairs of the lower allele of the CEPH sample 1347-02 (CEPH genomics repository) was subtracted from the size of the

microsatellite amplicon and used as a reference. SNP genotyping was carried using direct DNA sequencing (Applied BioSystems) or the Centaurus platform (Nanogen).

Statistical Methods for Association Analysis

For single marker association to type II diabetes, we used a likelihood ratio test to calculate a two-sided p-value for each allele. We present allelic frequencies rather than carrier frequencies for the microsatellites employed.

We calculated relative risk (RR) and population attributable risk (PAR) assuming a multiplicative model(*16, 17*). For the CEPH Caucasian HapMap data, we calculated LD between pairs of SNPs using the standard definition of D' (*21*) and R^2 (*22*). When plotting all SNP combinations to elucidate the LD structure in a particular region, we plotted D' in the upper left corner and p-values in the lower right corner. In the LD plot we present, the markers are plotted equidistantly rather than according to their physical positions.

RESULTS

Locus-wide association study

We previously reported genome-wide significant linkage to chromosome 5q for type II diabetes mellitus in the Icelandic population(10); in the same study, we also reported suggestive evidence of linkage to 10q and 12q. To follow up the 10q locus, we used an association approach employing a high density of genotyped microsatellite markers across a 10.5 Mb region (NCBI Build 34: Chr10:114.2 - 124.7 Mb) corresponding to this locus. We identified and typed 228 microsatellite markers -- i.e. to an average density of one marker every 46 kb (Table 1). All the markers were typed in 1185 Icelandic type II diabetes patients and 931 unrelated population controls.

Table 1: Location of the 228 genotyped microsatellites on chromosome 10 in NCBI Build 34 of the human genome assembly.

Alias	START: Build 34 Chr10 location	END: Build 34 Chr10 location
D10S1269	114186051	114186276
DG10S475	114389853	114390116
D10S168	114410102	114410266
DG10S478	114460845	114461228
DG10S479	114475488	114475632
DG10S480	114507574	114507829
DG10S481	114542657	114542924

Alias	START: Build 34 Chr10 location	END: Build 34 Chr10 location
DG10S1624	114545990	114546237
DG10S1625	114568323	114568715
DG10S488	114713594	114714008
DG10S1630	114770344	114770609
DG10S1631	114778307	114778598
DG10S492	114811884	114812269
DG10S494	114852114	114852280
DG10S495	114879344	114879474
DG10S496	114919414	114919678
DG10S498	114964123	114964270
DG10S500	115024471	115024854
DG10S501	115045332	115045710
DG10S508	115241356	115241602
DG10S1634	115267106	115267460
DG10S512	115357290	115357439
DG10S514	115400157	115400338
DG10S17	115463773	115464048
DG10S1635	115519619	115519900
DG10S520	115536945	115537130
D10S554	115695920	115696071
D10S1237	115784580	115784977
DG10S535	115858565	115858720
D10S1158	115937134	115937433
DG10S1636	115966165	115966382
DG10S540	115983225	115983471
DG10S1637	116025219	116025491
DG10S542	116054130	116054255
DG10S1638	116062921	116063264
D10S1776	116140681	116140897
DG10S546	116141340	116141590
DG10S547	116173634	116173887
DG10S1639	116184720	116184898
DG10S548	116202775	116203174
DG10S550	116288175	116288560

Alias	START: Build 34 Chr10 location	END: Build 34 Chr10 location
D10S562	116304948	116305132
DG10S1640	116344030	116344279
DG10S1641	116638155	116638540
DG10S566	116866173	116866431
D10S468	116869582	116869674
DG10S567	116904174	116904433
D10S1731	117001692	117001870
DG10S573	117070087	117070192
DG10S576	117153566	117153823
DG10S578	117196538	117196813
DG10S1644	117206992	117207391
DG10S579	117226056	117226234
DG10S580	117240674	117240858
DG10S584	117336471	117336821
DG10S585	117364742	117364845
DG10S586	117385650	117385816
DG10S589	117481892	117482165
DG10S590	117508690	117508966
DG10S591	117520912	117521057
DG10S593	117567541	117567800
D10S1748	117589638	117589885
DG10S596	117629981	117630119
DG10S597	117654759	117654928
DG10S523	117691905	117692329
DG10S598	117691905	117692156
D10S1773	117708786	117708989
DG10S599	117713714	117714115
DG10S524	117713997	117714115
DG10S600	117742602	117743019
DG10S525	117742701	117742986
DG10S1250	117861226	117861405
DG10S604	117867801	117868010
DG10S1293	117932494	117932721
DG10S1144	117950298	117950606
DG10S609	118014503	118014752
DG10S610	118041410	118041787
DG10S1252	118085912	118086081
DG10S612	118092869	118093247
DG10S613	118126058	118126312
DG10S614	118150018	118150178
D10S544	118164684	118164979
D10S1683	118211053	118211180
D10S1657	118287426	118287695
D10S545	118299618	118299851

Alias	START: Build 34 Chr10 location	END: Build 34 Chr10 location
DG10S1649	118306954	118307121
D10S187	118317655	118317730
DG10S1295	118375973	118376205
DG10S624	118401694	118402073
DG10S1203	118440472	118440835
DG10S627	118514695	118515072
DG10S1650	118521021	118521210
DG10S1681	118522946	118523333
DG10S628	118553693	118553836
DG10S634	118566844	118567191
DG10S639	118712208	118712596
DG10S640	118743450	118743821
D10S221	118766458	118766560
DG10S1686	118766464	118766561
DG10S641	118788135	118788401
DG10S1651	118794961	118795267
DG10S1255	118834290	118834438
DG10S644	118857362	118857745
DG10S1652	118862172	118862311
DG10S1654	118954536	118954869
DG10S1688	118972583	118972717
DG10S1689	118987319	118987480
DG10S1690	119004704	119004986
D10S1425	119004742	119004920
DG10S651	119030166	119030595
DG10S1655	119044005	119044188
DG10S1691	119078576	119078943
DG10S1207	119094382	119094722
D10S1693	119109493	119109731
DG10S1258	119131611	119131788
DG10S656	119177278	119177672
DG10S1694	119177430	119177614
DG10S1695	119204432	119204655
DG10S657	119204769	119205174
DG10S658	119223917	119224102
DG10S1696	119243071	119243408
DG10S1657	119282299	119282586
DG10S1658	119290241	119290632
DG10S661	119305067	119305226
DG10S662	119317406	119317660
DG10S663	119330718	119331131
DG10S1699	119364904	119365188
DG10S665	119396863	119397144
DG10S1659	119412611	119412992
DG10S667	119448478	119448736

Alias	START: Build 34 Chr10 location	END: Build 34 Chr10 location
DG10S1701	119473676	119473914
D10S1236	119473739	119473870
DG10S669	119485378	119485552
DG10S670	119505799	119505905
D10S190	119510348	119510554
DG10S1702	119510362	119510479
DG10S1153	119526060	119526329
DG10S673	119606691	119606963
DG10S1305	119615268	119615484
DG10S675	119659153	119659532
DG10S1661	119663175	119663453
DG10S1662	119700563	119700948
DG10S1306	119703996	119704204
DG10S1663	119783538	119783739
DG10S1704	119783569	119783694
DG10S631	119788517	119788678
D10S1148	119803465	119803663
D10S1150	119803465	119803662
D10S503	119803476	119803653
DG10S632	119811193	119811621
DG10S681	119811347	119811621
DG10S633	119833701	119833987
D10S2473	119833724	119833869
DG10S682	119838539	119838806
DG10S683	119853558	119853862
DG10S684	119880412	119880572
DG10S685	119909682	119910062
DG10S686	119923527	119923790
DG10S687	119954835	119955083
DG10S1212	119972358	119972707
DG10S1261	119995566	119995727
DG10S1350	120004924	120005036
DG10S1	120030830	120031131
DG10S693	120100794	120101005
DG10S1263	120132349	120132528
D10S542	120417003	120417230
DG10S1664	120444685	120444808
DG10S1163	120506796	120507066
DG10S703	120538236	120538484
DG10S704	120570334	120570593
DG10S706	120642052	120642312
DG10S708	120699520	120699811
DG10S709	120723780	120724158
D10S1701	120849161	120849428

Alias	START: Build 34 Chr10 location	END: Build 34 Chr10 location
DG10S716	120893782	120894153
DG10S1669	120969521	120969659
DG10S720	121016792	121017048
D10S1792	121042408	121042574
DG10S722	121070320	121070693
DG10S1181	121101362	121101685
DG10S724	121117025	121117286
DG10S1670	121162511	121162898
DG10S726	121217327	121217580
DG10S1167	121247552	121247838
DG10S729	121283257	121283429
DG10S730	121318865	121319131
DG10S731	121342622	121342893
DG10S1278	121384227	121384464
DG10S734	121425229	121425633
DG10S735	121446549	121446695
DG10S1185	121466936	121467248
DG10S1129	121472295	121472600
DG10S1085	121494260	121494657
DG10S1327	121526700	121526830
DG10S1271	121559895	121560066
DG10S741	121638254	121638391
DG10S1087	121647884	121648273
DG10S1359	121713760	121713892
DG10S1120	121726128	121726519
DG10S1671	121750886	121750993
DG10S1673	121823695	121823925
DG10S749	121841816	121841997
DG10S1134	121901381	121901668
DG10S1674	121931406	121931809
DG10S755	121976143	121976435
D10S1757	121989325	121989539
D10S209	121995173	121995376
DG10S757	122029990	122030248
DG10S1283	122045222	122045429
DG10S1191	122071761	122072115
DG10S761	122141102	122141322
DG10S1678	122146312	122146535
DG10S762	122167889	122168135
DG10S763	122185793	122185925
DG10S1284	122207287	122207508
DG10S1137	122220809	122221073
DG10S766	122257534	122257929
DG10S767	122283871	122284250

Alias	START: Build 34 Chr10 location	END: Build 34 Chr10 location
DG10S1361	122318975	122319081
DG10S1680	122390160	122390294
D10S1230	122407279	122407403
DG10S772	122421708	122421845
DG10S775	122463781	122463941
DG10S777	122524358	122524547
DG10S779	122580228	122580603
DG10S784	122719087	122719236
D10S1483	122948181	122948324
D10S587	124728937	124729112

Single marker association analysis with the microsatellite markers identified association with DG10S478 (Table 2 and FIG. 1).

Table 2: DG10S478 Association to Type II Diabetes in Iceland

Allele	Affected freq (n=1185)	Control freq (n=931)	RR [95% CI]	Two sided *P*
0	0.636	0.724	0.67	2.1×10^{-9}
4	0.005	0.002	2.36	0.12
8	0.093	0.078	1.21	0.090
12	0.242	0.178	1.48	4.6×10^{-7}
16	0.022	0.015	1.53	0.076
20	0.001	0.003	0.39	0.17
X	**0.364**	**0.276**	**1.50 [1.31,1.71]**	$\mathbf{2.1\times10^{-9}}$

Six alleles are observed with this tetra-nucleotide repeat, with alleles 0, 8 and 12 accounting for 98% of chromosomes in the population controls. Allele 0 showed a protective association (Relative Risk (RR)=0.67; $P=2.1\text{x}10^{-9}$) relative to the other alleles combined. This *P*-value is two-sided and takes into account that some of the patients are related to each other. DG10S478 is located in intron 3 of the transcription factor 7-like 2 (*TCF7L2* - formerly *TCF4*) gene on 10q25.2. This marker is within a well defined LD block of 74.9kb (based on the CEPH Caucasian HapMap Phase II) that encapsulates part of intron 3, the whole of exon 4 and part of intron 4 (FIG. 1).

When DG10S478 was genotyped in the CEPH Caucasian HapMap families, it became clear that allele G of SNP rs12255372, is observed to be nearly perfectly correlated with allele 0 of DG10S478 ($r^2 = 0.95$, $P = 5.53\text{x}10^{-38}$), and allele T of rs12255372 is correlated with other alleles of DG10S478. Moreover, the risk conferred by

alleles 8 and 12 of DG10S478 do not differ ($P = 0.3$). Hence it is natural to collapse all the non-0 alleles of DG10S478 into a composite allele which will be referred to as allele X. Allele X has frequency of 27.6% and 36.4% in controls and patients respectively. Assuming a multiplicative model (*16, 17*), compared to the risk for non-carriers, allele X has an estimated RR of 1.50 per copy carried.

Replication of the DG10S478 association to Type II Diabetes

To verify the association of DG10S478 to type II diabetes, the microsatellite was genotyped in a Danish type II diabetes cohort of 228 cases and 539 controls. The Danish cohort was selected from the PERF (Prospective Epidemiological Risk Factors) study in Denmark (*19*). This female type II diabetes cohort had been diagnosed previously with type II diabetes. The association observed in Iceland was replicated (Table 3).

Table 3: DG10S478 Association to Type II Diabetes in Denmark

Allele	Affected freq (n=228)	Control freq (n=539)	RR [95% CI]	Two sided *P*
0	0.669	0.740	0.71	0.0048
4	0.002	0.004	0.59	0.62
8	0.070	0.048	1.49	0.091
12	0.239	0.190	1.34	0.032
16	0.020	0.018	1.12	0.78
X	**0.331**	**0.260**	**1.41 [1.11, 1.79]**	**0.0048**

The composite at-risk allele X has a frequency of 26.0% in controls and 33.1% in type II diabetes cases, giving an estimated RR of 1.41 ($P = 0.0048$).

Subsequently, the microsatellite was genotyped in a US Caucasian type II diabetes cohort of 361 cases and 530 controls from the PENN CATH study. This study is a cross sectional study of the association of biochemical and genetic factors with coronary atherosclerosis in a consecutive cohort of patients undergoing cardiac catheterization at the University of Pennsylvania Medical Center. Type II diabetes was defined as a history of fasting blood glucose ≥126mg/dl, 2-hour post-prandial glucose ≥200mg/dl, use of oral hypoglycemic agents, or insulin and oral hypoglycemic in a subject greater than age 40. The association observed in Iceland was also replicated in this population (Table 4).

Table 4: DG10S478 Association to Type II Diabetes in the United States

Allele	Affected freq (n=361)	Control freq (n=530)	RR [95% CI]	Two sided *P*
-4	0.001	0.000	-	-
0	0.615	0.747	0.54	3.3×10^{-9}
4	0.003	0.004	0.73	0.72
8	0.085	0.049	1.79	0.0029
12	0.256	0.180	1.57	1.2×10^{-4}
16	0.040	0.020	2.07	0.012
X	**0.385**	**0.253**	**1.85 [1.51, 2.27]**	**3.3×10^{-9}**

The composite at-risk allele X has a frequency of 25.3% in controls and 38.5% in type II diabetes cases, giving an estimated RR of 1.85 ($P = 3.3\text{x}10^{-9}$). Combining the results from all 3 cohorts using a Mantel-Haneszel model *(NOTE 3)* yields an overall two-sided *P* of $4.7\text{x}10^{-18}$.

The association of the composite at-risk allele to type II diabetes in three populations constitutes strong evidence that variants of the *TCF7L2* gene contribute to the risk of type II diabetes.

After establishing beyond doubt the association of the allele X to type II diabetes, we investigated the mode of inheritance more closely. The dominant model and recessive model can be rejected as the heterozygous carriers clearly have increased risk relative to the non-carriers ($P < 1 \text{ x } 10^{-6}$) and reduced risk compared to the homozygous carriers ($P <$ 0.0001). The multiplicative model provides a better fit, but there is evidence that the risk of the homozygous carriers relative to the heterozygous carriers is greater than that of the risk of the heterozygous carriers relative to the non-carriers. Table 5 provides model-free estimates of the relative risks of the heterozygous carriers and homozygous carriers compared to the non-carriers.

Table 5: Model-free estimates of the relative risks

	Genotype Relative Risk			
Cohort	**00**	**0X [95% CI]**	**XX [95% CI]**	**PAR**
Iceland	1	1.41 [1.17,1.70]	2.27 [1.70, 3.04]	0.21
Denmark	1	1.37 [0.98,1.90]	1.92 [1.13, 3.26]	0.17
USA	1	1.64 [1.23, 2.19]	3.29 [2.13, 5.07]	0.28
Combined	**1**	**1.45 [1.26, 1.67]**	**2.41 [1.94, 3.00]**	**0.21**

The three cohorts have similar population frequency for the at-risk allele, but the RR estimates vary; with the strongest effect seen in the US cohort and the weakest in the Danish cohort. While there is no reason for the RR to be identical in the cohorts, it is

noted that the differences in the estimated relative risks do not quite reach statistical significance ($P > 0.05$). Combining the results from the cohorts assuming common relative risks, the heterozygous carriers and homozygous carriers are estimated to have relative risks of 1.45 and 2.41 respectively compared to the non-carriers (Table 5). Assuming a population frequency of 26% for the at-risk allele, heterozygous and homozygous carriers make up 38% and 7% of the population respectively. Hence, this variant has enough predictive value to be of clinical use. The corresponding population attributed risk is 21%, which is substantial from a public health point of view.

It should also be noted that allele X is in excess in impaired fasting glucose (IFG) individuals (fasting serum glucose between 6.1 and 6.9 mM). The composite at-risk allele X has a frequency of 27.7% in 1393 controls and 37.1% in 278 IFG cases, giving an estimated RR of 1.54 ($P = 1.36 \times 10^{-5}$).

Association of SNP markers within exon 4 LD block of TCF7L2 with type 2 diabetes.

In Table 6 we list microsatellite and SNP markers residing within the exon 4 LD block of TCF7L2. The table contains publically available SNPs, as well as SNPs discovered by sequencing the entire LD block region. The table furthermore provides polymorphic microsatellite markers residing within the block.

Table 6. Polymorphic markers residing within the exon 4 LD block of TCF7L2 (between markers rs4074720 and rs7087006, positions in Build 34 co-ordinates: rs4074720 (B34: 114413084) - rs7087006 (B34: 114488013) = 74929bp. Sequence identification references are indicated as appropriate, referring in each instance to the SEQ ID number for the amplimer containing the polymorphism, and forward and reverse primers, as disclosed in the Sequence listing.

A. Public SNPs (including all HapMap ethnicities)

Public Alias	Chromosome 10 B34 location	Base Change	Sequence ID NO:
rs4074720	114413084	A/G	
rs4074719	114413145	C/T	
rs4074718	114413204	C/T	
rs11196181	114413605	A/G	
rs11196182	114414744	C/T	
rs4603236	114414765	G/T	
rs7922298	114414856	C/T	
rs17747324	114417090	C/T	
rs7901695	114418675	C/T	17-19
rs11196185	114420079	C/T	
rs4132115	114420083	A/C	

Public Alias	Chromosome 10 B34 location	Base Change	Sequence ID NO:
rs4506565	114420628	A/T	14-16
rs7068741	114420845	C/T	
rs7069007	114420872	C/G	
rs7903146	114422936	C/T	11-13
rs11196187	114424032	A/G	
rs7092484	114425520	A/G	
rs10885402	114426284	A/C	
rs12098651	114426306	A/G	
rs6585198	114426824	A/G	
rs7910244	114427209	C/G	
rs12266632	114429546	C/G	
rs6585199	114429758	A/G	
rs7896811	114431304	C/T	
rs6585200	114433196	A/G	
rs6585201	114433370	A/G	
rs4319449	114433993	G/T	
rs12220336	114434854	A/G	
rs7896091	114436550	A/G	
rs12354626	114437016	A/G	
rs7075199	114437307	C/G	
rs7904519	114438514	A/G	
rs13376896	114441336	A/C	
rs10885405	114442257	C/T	
rs10885406	114442311	A/G	
rs11196192	114446874	G/T	
rs6585202	114447390	C/T	
rs7924080	114451599	C/T	
rs7907610	114451677	A/G	
rs12262948	114452313	C/G	
rs12243326	114453402	C/T	8-10
rs12265110	114453606	C/T	
rs7077039	114453664	C/T	
rs11196198	114456472	A/G	
rs12775336	114459590	G/T	
rs7904948	114459672	A/T	
rs7100927	114460635	A/G	
rs11196199	114460704	A/G	
rs17685538	114462058	C/G	
rs11592706	114463573	C/T	
rs7081912	114463678	A/G	
rs7895340	114466112	A/G	23-25
rs11196200	114466525	C/G	
rs11196201	114467894	A/T	
rs11196202	114470254	A/G	
rs11196203	114470447	A/C	
rs11196204	114470518	A/G	
rs11196205	114471634	C/G	20-22
rs10885409	114472659	C/T	
rs12255372	114473489	G/T	5-7
rs12265291	114474827	C/T	
rs7904443	114475774	A/G	
rs11196208	114475903	C/T	
rs7077247	114476658	C/T	

Public Alias	Chromosome 10 B34 location	Base Change	Sequence ID NO:
rs11196209	114477314	A/G	
rs4077527	114477628	A/G	
rs12718338	114477634	C/T	
rs11196210	114478558	C/T	
rs7907632	114481823	A/G	
rs7071302	114482114	G/T	
rs12245680	114484778	C/T	
rs11196213	114486141	C/T	
rs4918789	114486394	G/T	
rs7085785	114487050	C/T	
rs7085989	114487326	A/G	
rs7087006	114488013	A/G	

B. Novel SNPs discovered and subsequently validated in the exon 4 LD block of TCF7L2 (amplimers below):

deCODE Alias	Chromosome 10 B34 location	Base Change	Sequence ID NO:
SG10S405	114418658	C/T	26-28
SG10S428	114421901	A/C	29-31
SG10S422	114457824	A/G	32-34
SG10S427	114463480	A/T	35-37
SG10S408	114466074	A/T	38-40
SG10S409	114471574	A/C	41-43
SG10S406	114471618	C/G	42-44
SG10S407	114473534	C/G	45-47

C. Polymorphic microsatellites within the exon 4 LD block of TCF7L2 (amplimers below):

Microsatellite	C10 B34 Start	C10 B34 End	Sequence ID NO:
DG10S2164	114460344	114460627	48-50
DG10S478	114460845	114461228	2-4
DG10S479	114475487	114475632	51-53

Table 7. Amplimers and primers for selected markers within the exon 4 LD block of TCF7L2

>DG10S478
TTCAGGCCATTGGTGTTGTATATATTTCAAGATTTGCTCACAGGTCCAAA
GCTTAACTTAAGCTCCCTGAGACATATCATAAAATATGATTTGGGGAAAA
ACCCTAATGGGCCATGATCAGAACATTATTATTCAACAAAGGATGAAATG
CTTAAGCCAAGATGGCCTTCTTTCTTTCTTTCTTTCTTTCTTTTTTTTTA
ATGAAAGTTGAGCAGACTCCCGTCCAACAGTTTTCAATGTAGGAATTCCC
ACAGCCCCATTTGATTGCAGTTTGTTGAAAAGTTTAATGTTTTTGTAGGC
AATTCATAATTTCCACATTGAACAGCCTGAGAGGAAGAGAGCTGGAGCCC
ACTGTTGTTTTTGTAGTGGGATGGTGGGAACTTT (SEQ ID NO:2)

Primers:

F: TTCAGGCCATTGGTGTTGTA (SEQ ID NO:3)
R: AAAGTTCCCACCATCCCACT (SEQ ID NO:4)

>rs12255372
TTGTCCCTTGAGGTGTACTGGAAACTAAGGCGTGAGGGACTCATAGGGGT
CTGGCTTGGAAAGTGTATTGCTATGTCCAGTTTACACATAAGGATGTGCA
AATCCAGCAGGTTAGCTGAGCTGCCCAGGAATATCCAGGCAAGAAT
K
ACCATATTCTGATAATTACTCAGGCCTCTGCCTCATCTCCGCTGCCCCCC
CGCCCCCTGACTCTCTTCTGAGTGCCAGATTCAGCCTCCATTTGAATGCC
AAATAGACAGGAAATTAGCATGCCCAGAATCCACGTCTTTAGTGCACTCT
CTCCCCAGCTCCAAACCTGTTACTGCTTGTGTTCAACATCTCAGTAAAGC
TCAACAACATCGACCCATT (SEQ ID NO:5)

Primers:
F: TTGTCCCTTGAGGTGTACTGG (SEQ ID NO:6)
R: AATGGGTCGATGTTGTTGAG (SEQ ID NO:7)

>rs12243326
GCTGTGAAATCCCCTGTGTAGTGGGAAGAAGAAATAGCAAATCTTAGCTG
CCTTGGACCTGATATAATTATTTGTCTTCATTTACATGGTT
Y
ATCCTTCAAGGTTGAATAAATGATGTGGGAGCTAGTCAAGGGGCTTTAGG
TATGTGATTTCATGCCTACTTTTTTTTAGGTAGAGAAACTGAGGTCACAG
GGTACTAGAGAATGGACTCTAAGATTCAGGTTTCTGAATTGCCTGTGGTT
TTGTTGACTCAACTGCTCTTCTGTTGTTTTTTAGCCACATGCCTTGAAAC
AGTCCTCTTTCCCATGTTTCTTCATCAGCACCATTAACCCAAGGTATACT
GTCCTCTCTTATCTTTCACAAGGTCTTGGAGTTCCCATGCCTTTGTAAGC
ATCCCTCCCCGAGATTCAGCACCAACCAAAATCACATTTGGAAAAATTGC
TTGTTTCCCAAGAAGCTTTGGAGGATATGATTTTGTATAGAACGGGTTCA
CAGGTTTTCTGTTCATTCTTCTATGGTGGAGTGTGTGTGTATGTGACTCT
GTCTTCTCTCCATTCC (SEQ ID NO:8)

Primers:
F: GCTGTGAAATCCCCTGTGTAG (SEQ ID NO:9)
R: GGAATGGAGAGAAGACAGAGTCA (SEQ ID NO:10)

>rs7903146
AAGGGAGAAAGCAGGATTGAGCAGGGGGAGCCGTCAGATGGTAATGCAGA
TGTGATGAGATCTCTGCCGGACCAAAGAGAAGATTCCTTTTTAAATGGTG
ACAAATTCATGGGCTTTCTCTGCCTCAAAACCTAGCACAGCTGTTATTTA
CTGAACAATTAGAGAGCTAAGCACTTTTTAGATA
Y
TATATAATTTAATTGCCGTATGAGGCACCCTTAGTTTTCAGACGAGAAAC
CACAGTTACAGGGAAGGCAAGTAACTTAGTCAATGTCAGATAACTAGGAA
AAGGTTAGAGGGGCCCTGGACACAGGCCTGTGTGACTGAGAAGCTTGGGC
ACTTCACTGCTACATTTCATCTCTTCGCT (SEQ ID NO:11)

Primers:
F: AAGGGAGAAAGCAGGATTGA (SEQ ID NO:12)
R: AGCGAAGAGATGAAATGTAGCA (SEQ ID NO:13)

>rs4506565
CTGATGAGGGTAGGGAGCATCTGTCTGCAGCTTCATCTTCATTGTCTAGG
GGCTCCAGAAATATCTGTGAGTAAATAAGTTATTTAATCTTTGCCTCAAA
TTTCCAGTGACTGTAGGGATATAGCTGTGAGCCTCTAGGAGCTGAGATTT
TTTAAATTTCCCACTTAAACATTTATTTAAAAATTTTGTGCTCAGCATGG

ACTAAGGACTTTACATTCATTAACTCATTTACAGCTTGATCCTATGCGGT
GGGCATTCATTTACAGAGGATCCCATTTTACAGGTGAGGAAGAGGCCAGC
TAGGGGTGCAGCCTAGGTTAGTATTCTAGAGCTCATCAGGCTGTGTTGTC
CCCAGTGAAAGAATAAGCAAAGAAGTGAATGTTGTGCATTGAGAAAAATG
ACTCTCGGAGGAGGATGAGCCTCTCGGATATGGCGACCGAAGTGAT
W
TGGGGCCCTTGTCAAGGGTCTCTATTATGGCATCAAGAAAAGATGCTGCT
TTCGGTGATGCCCGAGGAGAGCCTCAATATTTTACATGGGAAACCTAAAA
AAGGGGCCATGTTGTGGTCTCTGCACCTAAGA (SEQ ID NO:14)

Primers:
F: CTGATGAGGGTAGGGAGCA (SEQ ID NO:15)
R: TCTTAGGTGCAGAGACCACAAC (SEQ ID NO:16)

>rs7901695
TATTTAGAAACCATAAAATCCACCTATTTGAGGTGTACAATTGAGTGATT
TTCTGTATAGTCACAGATCTGTGCAGTCATCCACACCCTCTAACTCCAGG
ACATTTTCCTCACCCCCGAGGAGAAACCTCCCTTACCCATTAGCAGTCAC
TCCTCATTTCCTCTCCCCCCAGCCCCTGGCAATCACTGTGGATTTGCCTG
TTCTTGACATTTCATATAAATGGTATCATAAAATCTA
Y
GGGCTTTTGTGTCTGTCTGCTTTCACTTAGCATACGGTTCTCAAGGTTCA
TCCAGTATTGTAGCATCTATCAGTATGTCATTCCTTTTTATGGCCAAATA
ATATTTTATTGTATGGATAGACATTTTGTTTATTCATTTATCTGTTTTTG
GTTATTATGAGTAACACTACTATGAACATTTTGCACAAATTTTTGTATTG
ACATGTTTTCATTTCTCCTGGGTATAGTCCTATGAGTGGAATTGCTGG
(SEQ ID NO:17)

Primers:
F: TATTTAGAAACCATAAAATCCACCTAT (SEQ ID NO:18)
R: CCAGCAATTCCACTCATAGGAC (SEQ ID NO:19)

>rs11196205
TTGTCTCCTTTTGTTTCTGCTACTGTGAATGATCCTGTGATGATCATCTT
TGTGTGTAAATCTTTGTCCCCTCGCCCCCTCCCCTTTTATTATTTTCTTG
GGATAGACCCCAGGACAAAAGGTAGAAAAGAACAAAGTGTTAAAAAATTT
CTTGATACATAGCCACAGATTATTTTCCTGAAAGTTCTCAACATTTATAA
CTAC
S
AGCAGTATGTAAGAGAGTTATGGTTGGAATGATTTTAATGTCTCTGGGGA
ATTTAACAACAAAAAAACTTTAGGCTTCTTTGGAGAGAGACATGCCCTTA
ACTCCACCCCGCCCTAGAACAGAGACCCAGCCCATCCAAGTCAGCCTCCC
CAGGTCCTCCACCTTCAAAACAGGCAAACGAAATCATTTCTTGAATAATT
GGTAGGCTTCAAGGTCAGATGTT (SEQ ID NO:20)

Primers:
F: TTGTCTCCTTTTGTTTCTGCTAC (SEQ ID NO:21)
R: AACATCTGACCTTGAAGCCTAC (SEQ ID NO:22)

>rs7895340
TCAGGGACAGTGCATAGGTGTAAAGAAGTTGCTGGTTGGGGGTTCTAATG
CAGGTTTCTCCAAAAGTGAATGCCCTGTTAAAAAAAAAATTCTTAACAAAT
ATACAGAGATTTTTTTTTTAAAAAAGTGTGACAGTTCTAGACACCTAGAG
AGTAAA
R
TGAAGAAGCCTGTTTTCAGGTTTCCCGCCTCCCTGAATTTCCCAGCATGG
TCCAGGCTTTGAAATTTATTTATCTGCTTTTGGCAATGGTTGATGGGAAT
TTCCCACATTTATTTTTTAGCTACAGAGAAAGGACATTATCTTTAAAATC

TCTTCGTTGTTCTCTCTCTTTGA (SEQ ID NO:23)

Primers:
F: TCAGGGACAGTGCATAGGTG (SEQ ID NO:24)
R: TCAAAGAGAGAGAACAACGAAGA (SEQ ID NO:25)

>SG10S405
TATTTAGAAACCATAAAATCCACCTATTTGAGGTGTACAATTGAGTGATT
TTCTGTATAGTCACAGATCTGTGCAGTCATCCACACCCTCTAACTCCAGG
ACATTTTCCTCACCCCCGAGGAGAAACCTCCCTTACCCATTAGCAGTCAC
TCCTCATTTCCTCTCCCCCCAGCCCCTGGCAATCACTGTGGATTTGCCTG
TTCTTGACATTTCATATAAA
Y
GGTATCATAAAATCTATGGGCTTTTGTGTCTGTCTGCTTTCACTTAGCAT
ACGGTTCTCAAGGTTCATCCAGTATTGTAGCATCTATCAGTATGTCATTC
CTTTTTATGGCCAAATAATATTTTATTGTATGGATAGACATTTTGTTTAT
TCATTTATCTGTTTTTGGTTATTATGAGTAACACTACTATGAACATTTTG
CACAAATTTTTGTATTGACATGTTTTCATTTCTCCTGGGTATAGTCCTAT
GAGTGGAATTGCTGGGTCATATAATAAATAACTGTTTAACATTTTGGGGA
GCTGCCAAACTTTTAAAACCTTGGGTTCTGTGATGTACCAGTTGTGTTAG
GCA (SEQ ID NO:26)

Primers:
F: TATTTAGAAACCATAAAATCCACCTAT (SEQ ID NO:27)
R: TGCCTAACACAACTGGTACATC (SEQ ID NO:28)

>SG10S428
TGCCAGGGGTTTTATGGTTAATTTTCCTCCATTATGAGGGTTGACTCAGC
CTTGGGTATTAGATGTCTTTGAGAATCCAGGGTTCAAATACCACAGCTGG
TAGAATGTTTCTCAACTTGGAGCCAATCTCCATCTACTGAAGGTACGCTG
GTTTAGACAGACAACAGGGACATCAGCATTTTAAAAAGCGGTGGAAAAAG
TTTGCTTGTCTTGATTGGAGCCATGACATTTTATTTTGAAATTTCAAATA
ACATGAAGGGAGGTTTGGAGCGGTTTTTGGTTTATCCAAAGGGCAGTGGA
TTGAAGGCTGAGAAACACCAGGCTGAATGGGAGAGGGGTTGGGGTCCCCC
TGTGAGATAGTGAAACAATGGTAGTGCCATCCAATGATAGGCACTTTTCT
GTCATTCAGAAGCAGAAAGGGGGCCAGAGGCCCATTGGCCTTACTGGG
M
AGTAAGCTGTAGAGCTGCTGCCTTTTCGTGAAAGGGTTGACACCAACCTT
CTCCCCCAGGAAGAGTGACCAGGGACCTGAGGGGCATGGTCGAGCAGATG
ACAGCCTTTGTAAAACATCTCC (SEQ ID NO:29)

Primers:
F: TGCCAGGGGTTTTATGGTTA (SEQ ID NO:30)
R: GGAGATGTTTTACAAAGGCTGTC (SEQ ID NO:31)

>SG10S422
TTGGTAGAGATGGGGTCTCCTAGGCTGGTCTTGAACTCCTGG
R
CTCAAGCAATCTTCCTGCCTCAGCCTTCCAAAGTACTGGGATTACTGGCG
TGGGCCACCATGCCTGGCTTGAAATTTTTCTATGGCTTTATTCTTTCTCC
AAGTACAGAGTCTACCCAACCTTCTGAGATCTTTGGTTTTCTTTTCCTAG
GTAACTATAGTACATACTTATTTATGTTAAACAACAGCAATCACACATTT
CTTTTTCTATACAGTCATGCTTTATAGGCAAATAAAGCCTCCGTCTTAGG
CTTTCTGGATTTTTTCAAAAGATGCAATTCCTGGAGTATGTTTTTACTTA
GAGCAAAGCAGCCTAGTCTCCTATACCTTCTGCATCTGCAGAAAAGTTGG
TTAAACAGACTTTGTAATGATGCCCCTTACAATTCTGAAGGGACTTGTGA

AATAGTTTCACAGAGTTTCAGTGTTAGGTATATTTGATCAATGCTAACTT
TTGGAAAACTTTGGTGCCTGTATGATTCAGAGGGTAGGGCAGAATATTAA
ATTAATCACAACTTCTTGTATTTTAACCATTCTGGGTAAATTGGGATTCC
GTGACGCCCAGGCAAAATTAT (SEQ ID NO:32)

Primers:
F: TTGGTAGAGATGGGGTCTCC (SEQ ID NO:33)
R: ATAATTTTGCCTGGGCGTCA (SEQ ID NO:34)

>SG10S427
TATCTTATATCCCCTCCAAGCATTCATTAACTGATGGATTAGTGAGTTGG
CCTTGAGAAGCATAAAGGCTCGTCTCCATGTGCTTCTAAGCATTGTGTCT
AAGTTCTGTTTGGTTTCCTGAGTGAAACTGTCTTAATGTTACCAACAGAA
GTTAAATGCCTAAGAG
W
TTCTTATACATGGGCTGAGTACCTCTGTGACTGGGCAAGCCACCTCACCT
CATTTTACCTTGTCTGCAAAATGAGGAACTGGGTCAACTCATCGTTCAAA
TCTCACTGAAAGCTAATTGATCGCTTTTGACAGAAGTAGCTCCCTTGGGC
CGTATATTTATTTCCTAGCTTGGAGGAAGGTGGGGACAGACAGAATTGAT
GTACACCTTTATTTTTATCTCTATGGTAAACCTGTGCATACTAAAGCATT
CCTCTGGTCTTTTGAGATGAGTGTATACATTGTGTCTGGCCCTGTGCATT
TTTTACCAAGAAGTAAGTTTTGTTGAGTAAACTTGGGTTGTATGAAGAAC
TGCATGCTCACCGTACTCAAGTAGCTTTTGCTACCTAAAGGACAGCTGCT
CATATGTACTTGACTTCCTTTAAAGTGAAGGATGATGACATTTGAAAAAC
GGAGGTTGAAAAGGAG (SEQ ID NO:35)

Primers:
F: TATCTTATATCCCCTCCAAGCATTC (SEQ ID NO:36)
R: CTCCTTTTCAACCTCCGTTTT (SEQ ID NO:37)

>SG10S408
TTGAGCATGTGTTATTTAATGAGTTATACCTCTGTCATATGTGTGTGTTT
ATATCACAAAATAACTTATTTTTATAAAACCATATTTTGAGTCATCATTT
GTGACAATGTCTTCTTTTCTCTGGTATAAATGAGGCATGTAGAAAGAAGA
TTGACATTTGCTAGAAGCTTCCCCTTTCCTCTAACTCCACAATAAAATGG
ATGCTCATAATTACATCTGCTCCTATAAGGTCAAGATTTCAGGGCTGGAA
GTGACCTTAGATCATTTAGGCCCAACTTGCCCTCAGGAAAGGAAACTGAG
GCCCAGAGATGCCTTAAGTGAATTGCCCAATGTCACACGCTGAGTCAGTG
GCCAGAGCAAGGCTTGGATCCAGTTCTCTGCTCCCTTTCCAGAGCCTTGT
GATGTCTTCTCTCCTACAGGAGGTGAAAATAACTGCTGTGGCTGGTTCTG
TTTTGCTGACTGTAAATTGGGTCATGGTCAGGGACAGTGCATAGGTGTAA
AGAAGTTGCTGGTTGGGGGTTCTAATGCAGGTTTCTCCAAAAGTGAATGC
CCTGTTAAAAAAAAATTCTTAACAAATATACAGAGATTTTTTTTT
W
AAAAAAGTGTGACAGTTCTAGACACCTAGAGAGTAAAGTGAAGAAGCCTG
TTTTCAGGTTTCCCGCCTCCCTGAATTTCCCAGCATGGTCCAGGCTTTGA
AATTTATTTATCTGCTTTTGGCAATGGTTGATGGGAATTTCCCACATTTA
TTTTTTAGCTACAGAGAAAGGACATTATCTTTAAAATCTCTTCGTTGTTC
TCTCTCTTTGAGTGAGGAGAGAAGATGTGAATCCTGGCAGTGGTTCAGAG
TGGACACAGCCCCTGTGTTTGTGGCATAGGCTCTGTGGGCCCCATGCCAG
GGAGCAGTACCCCCGTGTAAAGGAGTGGGGGTTTGTCCATTTGGATAGAG
CAAAGATCCTCCACCTCAAATCCCACAAGAACAGTTGCCACAACCTGGGC
CCTAAGCATCTCATTTTCCTATGTAGAAATTAATGATCTGGAGGAGATGG
CAAAACATTCCTTCCAGAGCCTGTGTGGATTTTGG (SEQ ID NO:38)

Primers:

F: TTGAGCATGTGTTATTTAATGAGTTA (SEQ ID NO:39)
R: CCAAAATCCACACAGGCTCT (SEQ ID NO:40)

>SG10S409
TAGTGCTCAGTATTTCCAACGTTCTGTTTATTTAAGATGAAAATTGCTGT
AGTTAATAAGCACTTCCCCATGTCATTAAAATGCTTAAGGATTTTTAATG
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GAACAAAGTGTTAAA
M
AATTTCTTGATACATAGCCACAGATTATTTTCCTGAAAGTTCTCAACATT
TATAACTACGAGCAGTATGTAAGAGAGTTATGGTTGGAATGATTTTAATG
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TCAGCCTCCCCAGGTCCTCCACCTTCAAAACAGGCAAACGAAATCATTTC
TTGAATAATTGGTAGGCTTCAAGGTCAGATGTT (SEQ ID NO:41)

Primers:
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R: AACATCTGACCTTGAAGCCTACC (SEQ ID NO:43)

>SG10S406
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GAACAAAGTGTTAAAAAATTTCTTGATACATAGCCACAGATTATTTTCCT
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S
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CATTTCTTGAATAATTGGTAGGCTTCAAGGTCAGATGTT (SEQ ID NO:44)

Primers:
F: TAGTGCTCAGTATTTCCAACGTTCT (SEQ ID NO:42)
R: AACATCTGACCTTGAAGCCTACC (SEQ ID NO:43)

>SG10S407
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ATACTTTGAGAGGGCCTTTGAAAGCCTTAGACAGATCATATCACAGACCT
CTATACTGAAAGTCGGG (SEQ ID NO:45)

Primers:
F: TGCTATGTCCAGTTTACACATAAGG (SEQ ID NO:46)
R: CCCGACTTTCAGTATAGAGGTCTG (SEQ ID NO:47)

>DG10S2164
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Primers:
F: CCATCTGTGGAGCAGAGTCA (SEQ ID NO:49)
R: TTCCATCAGCAGCAAGAATG (SEQ ID NO:50)

> DG10S479
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Primers:
F: TCCACGCAGAGAGGATCTAAA (SEQ ID NO:52)
R: GAGGGTCCTCCATTGAGTCG (SEQ ID NO:53)

To further investigate the possibility that other marker alleles in the exon 4 LD block of TCF7L2 exhibit a higher correlation with type II diabetes than allele X, we used the DG10S478 genotype data generated in the HapMap CEU samples. The five SNPs from HapMap Phase I with strongest correlation to DG10S478 were, in descending order, rs12255372 ($r^2 = 0.95$), rs7903146 ($r^2 = 0.78$), rs7901695 ($r^2 = 0.61$), rs11196205 ($r^2 = 0.43$), and rs7895340 ($r^2 = 0.42$). We genotyped these five SNPs in the three cohorts and the correlations between the five SNPs and DG10S478, the latter treated as a biallelic marker, were very similar to that observed in the CEU samples. All five SNPs showed association to type II diabetes. While some SNPs showed slightly higher estimated relative risks and lower p-values in one or two of the cohorts, none exhibited stronger association to type II diabetes than DG10S478 when the results for all three cohorts were combined using the Mantel-Haenszel model. However, although rs11196205 and rs7895340 clearly have weaker association to type II diabetes, compared to allele X (RR $= 1.56$, $P = 4.7\times10^{-18}$), the strength of the association to type II diabetes for allele T of

rs12255372 (RR = 1.52, $P = 2.5\times10^{-16}$) and for allele T of rs7903146 (RR = 1.54, $P = 2.1\times10^{-17}$) are comparable.

Following the subsequent release of HapMap Phase II in October 2005, two additional SNPs were identified that show strong correlation to microsatellite DG10S478 - rs12243326 (r^2=0.961) and rs4506565 (r^2= 0.716). The alleles associated with susceptibility to type 2 diabetes will be C for rs12243326 (C/T SNP) and T for rs4506565 (A/T SNP).

It should be noted that among those haplotypes that carry the C allele of rs7903146, those that carry the A allele of rs10885406 have an estimated relative risk of 1.06 compared to those that carry the G allele of rs10885406, but the difference is not statistically significant (P = 0.22).

In an attempt to replicate and refine this association with type 2 diabetes, we genotyped DG10S478, rs12255372 and rs7903146 in a large additional Danish cohort, consisting of 1111 cases and 2315 controls and in a more genetically diverse West African cohort, consisting of 618 cases and 434 controls derived from the Africa America Diabetes Mellitus study(*23*). In the Danes, all three variants were strongly associated with disease risk, as previously observed in Iceland. However, the association of allele T of rs7903146 (Relative Risk = 1.53, $P = 4.06\text{x}10^{-14}$, PAR = 24.4%) was noticeably stronger than that provided by the other two variants. In the West African study group, after adjustment for relatedness and ethnic origin, we replicated the association of allele T of rs7903146 to type 2 diabetes (Relative Risk = 1.45, 95% C.I. =1.20-1.76, $P = 0.000146$, PAR = 22.2%), but not in the case of the other two variants. This suggests that allele T of rs7903146 is either the risk variant itself or the closest known correlate of an unidentified risk variant. The exclusion of the markers DG10S478 and rs12255372 as at-risk markers in the West African group was possible because unlike in populations of European ancestry, where the T allele of rs7903146 occurs almost exclusively on chromosomes carrying both allele X of DG10S478 and allele T of rs12255372, in West Africans the T allele of rs7903146 occurs with both alleles of DG10S478 and rs12255372. This is consistent with the observation that T is the ancestral allele of rs7903146, whereas allele X of DG10S478 and allele T of rs12255372 are both different from the chimpanzee reference sequence. More generally, this finding is also consistent with the expectation that relatively diverse populations, such as those of West Africa, provide the means to

refine association signals detected in regions of strong linkage disequilibrium in more homogeneous populations.

DISCUSSION

In this study we describe the identification of a novel candidate gene for type II diabetes within the previously reported 10q linkage region(*10*), encoding transcription factor 7-like 2 (*TCF7L2* – formerly *TCF4*) on 10q25.2. We show that it confers risk of type II diabetes in Iceland, Denmark and the US with similar frequency and relative risks. While the variant does not explain a substantial fraction of the familial clustering of type II diabetes, the population attributed risk of at least 20% is significant from a public health point of view. Compared to the non-carriers, the relative risks of heterozygous carrier of the at-risk composite allele (approximately 38% of the population) and homozygous carriers (about 7% of the population) are 1.45 and 2.41, respectively. Hence, this variant has enough predictive value to be of clinical use.

We report the variant as a type II diabetes-associated microsatellite, DG10S478, within the third intron of the *TCF7L2* gene. The *TCF7L2* gene product is a high mobility group (HMG) box-containing transcription factor which plays a role in the Wnt signalling pathway. This pathway is considered one of the key developmental and growth regulatory mechanisms of the cell; it is mediated by secreted glycoproteins, known as Wnts, which initiate many signalling cascades within target cells upon binding to a cognate receptor complex, consisting of a member of the Frizzled family and a member of the LDL receptor family, Lrp5/6(*24*). Wnt signaling uncouples the central player in this pathway,β-catenin, from the degradation complex and translocates it to the nucleus where it transiently converts TCF factors from repressors into transcriptional activators(*25*). The β-catenin protein is also important for mediating cell adhesion through its binding of cadherins(*15*).

The NCBI RefSeq for *TCF7L2* contains 14 exons. However, Duval *et al*(*26*) showed that *TCF7L2* has 17 exons, of which 5 are alternative; in addition, it was reported that three alternative splice acceptor sites are used. This study also demonstrated the alternative use of three consecutive exons located in the 3' end of the *TCF7L2* gene which change the reading frames used in the last exon, leading to the synthesis of a large number of *TCF7L2* isoforms with short, medium, or long COOH-terminal ends..

Similar to TCF7L2, five of the six positionally cloned genes for the rare Mendelian forms of Type II Diabetes, namely maturity-onset diabetes of the young (MODY), are transcription factors(*27*). Additional transcription factors have been implicated in the pathogenesis of type II diabetes, including peroxisome proliferator-activated receptor gamma (PPARγ)(*7*) and the forkhead gene family(*28, 29*). Noble *et al* described a missense mutation (C883A) in the related *TCF7* gene in type 1 diabetes(*30*). However, it is not clear if *TCF7* and *TCF7L2* operate in the same pathway with respect to the pathogenesis of diabetes.

Mutations have been described in the *TCF7L2* gene, including the deletion of an A in an (A)9 coding repeat (exon 17)(*26, 31-33*) and a number of mutations in colorectal cell lines(*26*). DG10S478 resides within a clearly defined 74.9kb LD block (CEPH Caucasian HapMap Phase II) that encapsulates exon 4 and flanking intronic sequences 5' and 3' to the exon . It is possible that DG10S478 is the causative variant itself; it is also possible that DG10S478 is a surrogate for an underlying variant that affects transcription, splicing or message stability. Such a variant is likely to be in strong LD with DG10S478, i.e. the variant resides within the exon 4 LD block of TCF7L2

Several lines of evidence suggest an enteroendocrine role of this gene in the pathogenesis of type II diabetes. Firstly, TCF7L2 has been implicated in the development of colorectal cancer(*34*) and small-molecule antagonists of the oncogenic TCF/β-catenin protein complex have been already described(*35*). In addition, *TCF7L2–/–* mice, which die within 24 hours after birth, lack an intestinal epithelial stem-cell compartment(*36*). Variants of the *TCF7L2* gene could influence the susceptibility to type II diabetes through altering levels of the insulinotropic hormone glucagon-like peptide 1 (GLP-1), one of the peptides encoded by the proglucagon gene whose expression in enteroendocrine cells is transcriptionally regulated by TCF7L2. In concert with insulin, GLP-1 exerts crucial effects on blood glucose homeostasis(*12*). GLP-1 analogs and inhibitors of dipeptidyl peptidase IV are currently in clinical development.

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

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CLAIMS:

1. A method of diagnosing a susceptibility to type II diabetes in an individual, comprising analyzing *in vitro* the individual for a marker or haplotype within the exon 4 LD block of TCF7L2, between markers rs4074720 and rs7087006, wherein the marker or haplotype is in strong linkage disequilibrium, characterized by numerical values of r^2 greater than 0.2, with at least one marker selected from DG10S478, rs12255372, rs7895340, rs11196205, rs7901695, rs7903146, rs12243326 and rs4506565, and, wherein the presence of the marker or haplotype is indicative of a susceptibility to type II diabetes.

2. The method of Claim 1, wherein the marker or haplotype comprises at least one marker selected from the markers listed in Table 6, or at least one marker in strong linkage disequilibrium, characterized by numerical values of r^2 greater than 0.2, with at least one marker listed in Table 6.

3. The method of Claim 2, wherein the marker or haplotype comprises at least one marker selected from the markers listed in Table 6.

4. The method of any of the preceding Claims, wherein the marker or haplotype is a marker.

5. The method of any of the preceding Claims, wherein the marker or haplotype is indicative of increased susceptibility of type II diabetes.

6. The method of Claim 5, wherein the increased susceptibility is characterized by a relative risk of at least 1.2, including a relative risk of at least 1.3 and a relative risk of at least 1.4.

7. The method of any of the Claims 1-4, wherein the marker or haplotype is indicative of decreased susceptibility of type II diabetes.

8. The method of claim 6, wherein the decreased susceptibility is characterized by a relative risk of less than 0.8, including a relative risk of less than 0.7.

9. The method of any of the Claims 4 - 6, wherein the marker is selected from the group consisting of DG10S478, rs12255372, rs7895340, rs11196205, rs7901695, rs7903146, rs12243326, and rs4506565, and wherein the presence of a non-0 allele in DG10S478, a T allele in rs12255372; an A allele in rs7895340; a C allele in rs11196205; a C allele in rs7901695; a T allele in rs7903146; a C allele in rs12243326; or an T allele in rs4506565, is indicative of increased susceptibility to type II diabetes.

10. The method of Claim 9, wherein the marker is rs7903146, and wherein the presence of a T allele in rs7903146 is indicative of increased susceptibility to type II diabetes.

11. The method of Claim 4, 7 or 8, wherein the marker is selected from the group consisting of DG10S478, rs12255372, rs7895340, rs11196205, rs7901695, rs7903146, rs12243326, and rs4506565, and wherein the presence of a 0 allele in DG10S478, a G allele in SNP rs12255372; a G allele in rs7895340; a G allele in rs11196205; a T allele in rs7901695; a C allele in rs7903146; a T allele in rs12243326; or an A allele in rs4506565 is indicative of a decreased susceptibility to type II diabetes.

12. The method of Claim 11, wherein the marker is rs7903146, and wherein the presence of a C allele in rs7903146 is indicative of decreased susceptibility to type II diabetes.

13. A method of assessing an individual for probability of response to a TCF7L2 therapeutic agent, comprising: detecting *in vitro* a marker within the exon 4 LD block of TCF7L2, between markers rs4074720 and rs7087006, wherein the marker or haplotype is in strong linkage disequilibrium, characterized by numerical values of r^2 greater than 0.2, with at least one marker selected from DG10S478, rs12255372, rs7895340, rs11196205, rs7901695, rs7903146, rs12243326 and rs4506565, and, wherein the presence of the marker is indicative of a probability of a positive response to a TCF7L2 therapeutic agent.

14. The method of Claim 13, wherein the marker is selected from the group consisting of DG10S478, rs12255372, rs7895340, rs11196205, rs7901695, rs7903146, rs12243326, and rs4506565.

15. The method of Claim 14, wherein the marker is marker rs7903146, and wherein the presence of a T allele in rs7903146 is indicative of a probability of a positive response to a TCF7L2 therapeutic agent.

16. The method of any of the Claims 13 - 16, wherein the TCF7L2 therapeutic agent is an agent selected from the group set forth in the Agent Table.

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GRANT, Struan F. A.

<120> GENETIC VARIANTS IN THE TCF7L2 GENE AS DIAGNOSTIC MARKERS FOR RISK OF TYPE 2 DIABETES MELLITUS

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atgattaaac cccaatttac tgaatcaatg ccatattgtt gggtctttag attgtctcct 180

tttgtttctg ctactgtgaa tgatcctgtg atgatcatct ttgtgtgtaa atctttgtcc 240

cctcgccccc tccccttta ttatttcctt gggatagacc ccaggacaaa aggtagaaaa 300

gaacaaagtg ttaaamaatt tcttgataca tagccacaga ttatttcct gaaagttctc 360

aacatttata actacgagca gtatgtaaga gagttatggt tggaatgatt ttaatgtctc 420

tggggaattt aacaacaaaa aaactttagg cttctttgga gagagacatg cccttaactc 480

cacccegccc tagaacagag acccagccca tccaagtcag cctccccagg tcctccacct 540

tcaaaacagg caaacgaaat catttcttga ataattggta ggcttcaagg tcagatgtt 599

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<212> DNA
<213> Homo sapiens

<400> 42
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<210> 43
<211> 23
<212> DNA
<213> Homo sapiens

<400> 43
aacatctgac cttgaagcct acc 23

<210> 44
<211> 599
<212> DNA
<213> Homo sapiens

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cctcgccccc tccccttta ttatttcctt gggatagacc ccaggacaaa aggtagaaaa 300

gaacaaagtg ttaaaaaatt tcttgataca tagccacaga ttattttcct gaaagttcts 360

aacatttata actacgagca gtatgtaaga gagttatggt tggaatgatt ttaatgtctc 420

tggggaattt aacaacaaaa aaactttagg cttctttgga gagagacatg cccttaactc 480

cacccegccc tagaacagag acccagccca tccaagtcag cctccccagg tcctccacct 540

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ctgscccccc gcccccctgac tctcttctga gtgccagatt cagcctccat ttgaatgcca 180

aatagacagg aaattagcat gcccagaatc cacgtcttta gtgcactctc tccccagctc 240

caaacctgtt actgcttgtg ttcaacatct cagtaaagct caacaacatc gacccattac 300

ttaggcctca aaccttgggt ggcatcgtcg attgctcttt tctttcatac cccacattca 360

acccatcagc ccatcccaca ggcccaagtg tgtcctctct accttcaaag cgtgtgtggc 420

atccaccgct tatcaccacc tctgccatta ccactggagt ccagtgccat catctctcac 480

ttggatgtgg ccagagtgtc tttgctggtc tccttcttgc ttcctacctt tgtaacagcc 540

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ttagacagat catatcacag acctctatac tgaaagtcgg g 641

<210> 46
<211> 25
<212> DNA
<213> Homo sapiens

<400> 46
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<210> 47
<211> 24
<212> DNA
<213> Homo sapiens

<400> 47
cccgactttc agtatagagg tctg 24

<210> 48
<211> 284
<212> DNA
<213> Homo sapiens

<400> 48
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ttctgcttgt ttagaaaaga aatttataca gagccccccag tagcagctgt aaggggggcag 240

gttcttggag cagcccatcc tcaacattct tgctgctgat ggaa 284

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<213> Homo sapiens

<400> 49
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<210> 50
<211> 20
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<213> Homo sapiens

<400> 50
ttccatcagc agcaagaatg 20

<210> 51
<211> 145
<212> DNA
<213> Homo sapiens

<400> 51
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accacacaca cacacacaca cacacacaca cacacacaca cagacacata catatgcaca 120

caccccgact caatggagga ccctc 145

<210> 52
<211> 21
<212> DNA
<213> Homo sapiens

<400> 52
tccacgcaga gaggatctaa a 21

<210> 53
<211> 20
<212> DNA
<213> Homo sapiens

<400> 53
gagggtcctc cattgagtcg 20