

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



# Genetic Determinants of Type 1 Diabetes

Mohamed M. Jahromi

*Salmaniya Medical Complex, Ministry of health,  
Kingdom of Bahrain*

## 1. Introduction

Type 1 diabetes is (T1D) is an autoimmune disorder characterized by the T-cell-mediated destruction of the insulin-secreting  $\beta$  cells of the pancreatic islet of Langerhans.

T1D is heterogeneous in terms of age at diabetes development with as many adults developing the disorder as children (Barnett et al 1981). Genetic susceptibility is dependent on the degree of genetic identity with the proband, and the risk of diabetes in families has a non-linear correlation with the number of alleles shared with the proband. The highest risk is naturally observed in monozygotic twins (100% sharing) followed by first, second, and third degree relatives (50%, 25%, 12.5% sharing, respectively) (Millward et al 1986).

About 18 regions of the genome have been linked with influencing T1D risk. These regions, each of may contain several genes. Over 40 genes and loci have been associated with T1D (Barett et al 2009) have been labeled *IDDM1* to *IDDM18*.

The most well studied is *IDDM1*, which contains the HLA genes that encode immune response proteins. Variations in HLA genes are an important genetic risk factor, but they alone do not account for the disease and other genes are involved. There is increasing evidence other MHC linked gene or loci with a potential impact on the risk to T1D exist (Renando et al 2001).

There are three other non-MHC genes which have been identified thus far. One of these non-MHC genes is the insulin gene, and the other non-MHC gene maps close to CTLA4, which has a regulatory role in the immune response and the third confirmed non-MHC gene is PTPN22 that is involved in modulating T-cell activation.

## 2. Global distribution of T1D

T1D is reaching epidemic proportions throughout the world. The incidence of T1D is rapidly increasing in specific regions T1D affects incidence is highly variable among different populations. The overall ratio of incidence of T1D varies from 0.1/100 000 per year in China to more than 36/100 000 per year in Sardinia and in Finland. This drastic variation, a more than 350-fold, among populations is analyzed (Kavvoura & Ionnisidis 2005). The polar equatorial gradient (i.e., north-south gradient) described for disease incidence is not as strong as previously thought (e.g. for example Sardinia with extremely high incidence). Most populations with very high incidence rates are European. Although it is well established that the incidents rate of T1D is as low as 0.1/100 000 amongst Asians (Kavvoura & Ionnisidis 2005) high incidence rates have also been noted in Kuwait (Shaltout et al 2002), Oman

(Abdullah 2005), Bahrain (Jahromi unpublished) and Puerto Rico (Karvonen et al 2000). In fact, a relatively high gradient risk has been reported among some non-Europid ethnicities (i.e., admixed partly African [1/100 000 per year in Mauritius versus 15/100 000 per year in Chicago] and Arab [5/100 000 per year in Sudan vs. 21/100 000 per year in Kuwait] (Shaltout et al 2002).

## 2.1 Pathogenesis

T1D develops slowly and progressive abnormalities in pancreatic  $\beta$  cell function herald what appears to be a sudden development of hyperglycemia. Rising HbA1c in the normal range (Stene et al 2006), impaired fasting or glucose tolerance, as well as loss of first phase insulin secretion usually precede overt T1D. The exact pancreatic  $\beta$  cell mass remaining at diagnosis is poorly defined and there is almost no studies of insulinitis prior to onset of T1D (Gianani et al 2006). For patients with long-term T1D there is evidence of some  $\beta$  cell function remaining (C-peptide secretion) though  $\beta$  cell mass is usually decreased to less than 1% of normal (Meier et al 2005). At present methods to image/quantitate  $\beta$  cell mass and insulinitis are only beginning to be developed. However, animal studies have provided the first methods to image islet mass utilizing a labeled amine (dihydrotetrabenazine) (Souza et al 2006). A number of techniques are being evaluated to image insulinitis.

An increasing body of evidence indicates that the development of T1D is determined by a balance between pathogenic and regulatory T lymphocytes (Jahromi et al 2010). A fundamental question is whether there is a primary autoantigen for initial T cell autoreactivity with subsequent recognition of multiple islet antigens. A number of investigators have addressed in the NOD mouse (spontaneously develops T1D) the importance of immune reactivity to insulin with the dramatic finding that eliminating immune responses to insulin blocks development of diabetes and insulinitis, and importantly immune responses to downstream autoantigens such as the islet specific molecule IGRP (Krishnamurthy et al 2006). Knocking out both insulin genes (mice in contrast to man have two insulin genes) with introduction of a mutated insulin with alanine rather than tyrosine at position 16 of the insulin B chain prevents development of diabetes (Nakayama et al 2005). Recognition of this B-chain peptide of insulin by T lymphocytes depends upon a “non-stringent” T cell receptor with conservation of only the alpha chain sequence (Valpha and Jalpha) and not the N-region of the alpha chain, or the  $\beta$  chain (Homann & Eisenbarth 2006).

Interestingly, a study of pancreatic lymph node from two patients with T1D found a conserved T cell receptor, with T cells reacting with insulin A chain peptide amino acids 1-15.

There are large scale studies such as T1DGC (Barrett et al 2009) (USA), Teddy (International), (Nakayama et al 2005), DAISY (Liu et al 2005), DIPP BabyDiab (Homann & Eisenbarth 2006) and where tens of thousands of infants have been HLA typed and followed from birth for the development of islet autoantibodies and then progression to diabetes. Although T1D rate is increasing in Arabian countries recently, however, centers with diabetes interest have been found in Kuwait, UAE, Qatar, Bahrain as well as Saudi Arabia that would help in case of international contribution in determining the T1D pathogenesis. Especially since there are likely changing environmental factors contributing to the world-wide increase in the incidence of T1D and the above studies, in addition to providing crucial information for prediction are searching for such factors.

## 2.2 Genetic of T1D

### 2.2.1 “Monogenic” inheritance

The immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked (IPEX) syndrome is caused by a mutation of the foxP3 gene, a transcription factor that controls the development of regulatory T cells is a cause of neonatal diabetes (Jahromi & Eisenbarth 2007).

As reflected in the name, children with disorder suffer from overwhelming autoimmunity and usually die as infants. Of note bone marrow transplantation can reverse disease. IPEX syndrome is rare, as is neonatal diabetes. In the differential diagnosis of neonatal diabetes it must be recognized that half of children developing permanent neonatal diabetes have a mutation of the Kir6.2 molecule of the sulfonylurea receptor. These children with their non-autoimmune form of diabetes can be treated with oral sulfonylurea therapy.

Though more common than IPEX syndrome, the APS-1 syndrome (Autoimmune Polyendocrine Syndrome Type 1) is also rare. It results from a mutation of the AIRE gene, another transcription factor (Jahromi & Eisenbarth 2007). Approximately 15% of patients with this syndrome develop autoimmune diabetes. The leading hypothesis as to etiology (e.g. Addison’s disease, mucocutaneous candidiasis, and hypoparathyroidism) is that AIRE controls expression of autoantigens and negative selection of autoreactive T lymphocytes within the thymus. A very recent dramatic discovery is the demonstration that essentially 100% of patients with APS-1 have autoantibodies reacting with interferon alpha and other interferons. Such autoantibodies are extremely rare and essentially not found in patients with T1D or Addison’s disease outside of the syndrome.

Patients with T1D and their relatives are at risk for development of thyroid autoimmunity, celiac disease, Addison’s disease, pernicious anemia and a series of other autoimmune disorders (Aly et al 2006). Approximately 1/20 patients with T1D have celiac disease by biopsy though the majority have no symptoms (Hoffenberg et al 2004). These asymptomatic individuals are usually detected with screening for transglutaminase autoantibodies. The level of transglutaminase autoantibodies relates to the probability of a positive biopsy and it is important for clinicians to know the threshold for likely positive biopsy for the assay they employ (Liu et al 2005). There remains controversy as to whether asymptomatic celiac disease when detected should be treated with a gluten free diet and large clinical trials are needed to address this question.

### 2.2.2 “Polygenic inheritance”

T1D has become one of the most intensively studied polygenic disorders. There are rare variants of immune mediated diabetes with single gene mutations including the APS-I syndrome (AIRE autoimmune regulator gene) and the IPEX syndrome (Fox P3 mutation) (Jahromi & Eisenbarth 2006). Multiple genetic factors influence both susceptibility and resistance to the common forms of T1D (Barrett et al 2009). Although a significant proportion of patients with T1D lack a family history of the disease, there is significant familial clustering with an average prevalence of 6% in siblings compared to 0.4% in the US Caucasian population. Of note, there is a 3.8 % risk of T1D in Japanese siblings of patients with T1D compared to 0.01~0.02 % prevalence in the general Japanese population (Hoffenberg 2004). The sibling ratio ( $\lambda_s$ ) can be calculated as the ratio of the risk to siblings over the disease prevalence in the general population, and thus  $\lambda_s = 6/0.4 = 15$  and  $3.8/0.01 \sim 0.02 = >100$  for the US and Japan respectively (Hoffenberg 2004).

Genetic susceptibility of different genes and loci have been identified using both association and linkage studies. Using the candidate gene approach, association studies provided

evidence for the first two susceptibility loci, the HLA region and the insulin gene (*INS*) locus. These two loci only contribute a portion of the familial clustering (40-50% for *HLA* and 10% for *INS*), suggesting the existence of additional loci (Aly et al 2006, Jahromi & Eisenbarth 2006 & 2007, Barrett et al 2009). The next most potent locus for T1D of man was also discovered using a candidate gene approach, namely the LYP (PTPN22) gene with an odds ratio of approximately 1.7 for a "missense" mutation that contributes to multiple different autoimmune disorders (. Recently, however, Cytotoxic T lymphocyte antigen-4 (CTLA-4) gene and SUMO-4 with weak association were described following genome analysis. For both the associations appear heterogeneous dependent on the population (e.g. stronger association of Sumo-4 in Asian populations and lack of association in multiple European populations) (Nielsen et al 2011) , and nature of the disease (e.g. CTLA-4 associated with diabetes with thyroid autoimmunity) (Meier et al 2005). Undoubtedly, large extensive control case studies will reveal more loci, each providing a piece to the genetic puzzle of T1D, but with difficulty of distinguishing false positive signals with very weak associations.

2.2.2A The Major Histocompatibility Complex (MHC)

The major loci for susceptibility to T1D are located within the HLA (Human Leukocyte Antigen) region on the short arm of chromosome 6 and provide up to 40-50% of the inheritable T1D risk(Jahromi & Eisenbarth 2006, Barrett et al 2009, Jahromi et al 2010, Neilson et al 2011).

2.2.2A1 The HLA classes

Within the MHC region, they are grouped into three classes. **Class I genes:** (HLA - A, HLA - B and HLA-C as major & HLA-D, HLA-E, and HLA-F as minor subclasses) (Marsh et al 2005) encode class I MHC antigens, located on the surface of all nucleated cells (Jahromi & Eisenbarth 2007). The HLA complex was first linked to T1D when associations with several HLA class I antigens (eg HLA-B8, -B18, and -B15) were discovered by serological typing and affected sib-pair analysis showed evidence of linkage(Todd et al 1989, Awata et al 1990). **Class II genes:** (DQ, DR, and DP in that order of risk) were shown to be even more strongly associated with the disease (Todd et al 1989, Mijovic et al 1991). HLA class II antigens are found exclusively on B-lymphocytes, macrophages, epithelial cells of the islets of pancreatic Langerhans and activated T-lymphocytes. Their expression on other cells may be induced by cytokines such as interferon (IFN) and tumor necrosis factor (TNF)- $\alpha$  (Jahromi et al 2000). With the development of typing reagents, HLA class II alleles. However, other loci within or near the HLA complex appear to modulate T1D risk, and add further complexity to the analysis of *IDDM1* -encoded susceptibility. **HLA Class III:** The TNF (Tumor Necrosis Factor) gene is a strong candidate from class III, Table 1.

|   |               |  |
|---|---------------|--|
| Major histocompatibility complex (MHC)/ Human leukocyte antigen (HLA) | MHC class I   | HLA-A • HLA-B• HLA-C • HLA-E • HLA-F • HLA-G (Bodmer et al 1992)   |
|   | MHC class II  | HLA-DM ( $\alpha$ , $\beta$ ) • HLA-DO ( $\alpha$ , $\beta$ ) • HLA-DP ( $\alpha$ 1, $\beta$ 1) • HLA-DQ ( $\alpha$ 1, $\alpha$ 2, $\beta$ 1, $\beta$ 2, $\beta$ 3) • HLA-DR ( $\alpha$ , $\beta$ 1, $\beta$ 3, $\beta$ 4, $\beta$ 5) (Marsh et al 2005) |
|   | MHC class III |  |

Table 1. Different classes of Major Histocompatibility Complex



The great majority of Caucasian patients have the HLA-DR3(with [DQA1\*0501, DQB1\*020]DQ2) or -DR4(with [DQA1\*0301,DQB1\*0302]DQ8) class II alleles and approximately 30% to 50% of patients are DR3/DR4 heterozygotes ( Barrett et al 2009). The DR3/DR4 (DQ2, DQ8) genotype confers the highest diabetes risk with a synergistic mode of action, Table 2 (Mitchel et al 2007). However, DR4/DR9 has been reported to be a highly susceptible haplotype in Japanese. The absence of DR3 haplotypes in Japanese population may contribute to lower frequency of the disease in Japan (Fain & Eisenbarth 2002). On the other hand, in Chinese population the DR3/DR9 genotype is highly susceptible (Xiao-mei et al 2009). In fact DQ-DR linkage disequilibrium patterns of HLA haplotypes in different populations may explain part of the world-wide differences in the frequency of incidence of T1D (e.g.DRB1\*0405-DQB1\*0302 is a high risk haplotype while DRB1\*0403-DQB1\*0302 is neutral or protective). In Arab population, however, patients with T1D have almost similar HLA genetic susceptibility as Caucasian patients, this may confirms the increase rate of incidents of T1D (Shaltout et al 2002, Al Abbasi et al 2002).

| Relative risk |    |
|---------------|----|
| HLA-DR3       | 5  |
| HLA allele    | 6  |
| HLA-DR3/DR4   | 16 |

Extracted from Mitchel et al 2007

Table 2. HLA and T1D with increased risk

Furthermore, DRB1\*1501-DQB1\*0602 is protective in all populations which have been studied to date (shaltout et al 2002, Caillat et al 1997, Escribano-De-Diego et al 1999, Kawasaki et al 2004). Analysis of rare “recombinant” haplotype suggests that DQB1\*0602 provides protection and not DRB1\*1501. Specific allelic combination of DR/DQ loci have been shown to determine the haplotypic risk for T1D, protective and susceptible. Table 4 have summarized the most susceptible and the most protective combinations. The comparison of closely related DR-DQ haplotype pairs

| DRB1 | DQA1 | DQB1 | Effect on T1D |
|------|------|------|---------------|
| 0405 | 0301 | 0302 | S1            |
| 0401 | 0301 | 0302 | S2            |
| 0301 | 0501 | 0201 | S3            |
| 0402 | 0301 | 0302 | S4            |
| 0404 | 0301 | 0302 | S5            |
| 0701 | 0201 | 0503 | P1            |
| 1401 | 0101 | 0503 | P2            |
| 1501 | 0102 | 0602 | P3            |
| 1104 | 0501 | 0301 | P4            |
| 1303 | 0501 | 0301 | P5            |

Haplotype frequency in either proband or control subjects, five most susceptible (S) and five most protective (P) haplotypes are indicated (Erlik et al 2008)

Table 3. Frequency distribution of DRB1-DQB1 haplotype

with different T1D risks allowed identification of specific amino acid positions critical in determining disease susceptibility, only a single change of an amino acid would change degree of genetic susceptibility drastically (Erlick et al 2008).

On the other hand, combined typing of high risk HLA alleles with analysis of HLA haplotype sharing can identify siblings with an extraordinary genetic risk of T1D. This strongly implicates major genes in addition to DR and DQ alleles. The current extensive MHC analysis and multiple studies of conserved haplotypes (eg. A1-B8-DR3-DQ2 common extended haplotype) (Alper et al 1992) conferring increased risk. Increasing studies have confirmed the significant association of HLA-F region in determining the risk for T1D (Al et al 2008, Barrett et al 2009, Brorsson et al 2009). Interestingly through out our large affected family based controls (AFBAC) we have found that there is significant signal between patients with T1D and controls in HLA-F region in particular that contributes to T1D risk which seems to go with DRB\*0401, but has an independent risk. In other words this signal may have certain link with the possible candidate gene susceptible to T1D other than DQ/DR (Jahromi in process).

### 2.2.2B Non MHC genes

There are about 40 non MHC genes or loci which are candidates for genetic susceptibility to T1D (Barrett et al 2009). The most well known are discussed in this chapter.

#### 2.2.2B1 Insulin gene

Insulin is composed of two distinct polypeptide chains, chain A and chain B, which are linked by disulfide bonds. Many proteins that contain subunits, such as hemoglobin, are the products of several genes. However, insulin is the product of one gene, *INS*. The research done by Nakayama and coworkers have strongly shown that insulin is a primary autoantigen in the beginning stages of diabetes (Nakayama et al 2005).

Also, supporting this evidence is the presence of insulin antibodies in the blood of prediabetic and diabetic patients. The insulin gene is the second well established susceptibility locus in T1D on chromosome 11p 15.5 (Jahromi & Eisenbarth 2006).

The 4.1 Kb region containing the insulin gene (*INS*) and its flanking regions contain several polymorphisms in linkage disequilibrium (Stead et al 2000) that have been associated with diabetes risk. All the polymorphisms identified within this region lie outside coding sequences, confirming that diabetes susceptibility must derive from genetic influences on the expression of the insulin gene. Extensive studies involving polymorphisms in the neighboring *HUMTHO1* (tyrosine hydroxylase) and *IGF2* genes provided strong evidence that *INS* is the main susceptibility determinant in this region (Vardi et al 1988, Stead et al 2000). Shortly after its discovery (Vardi et al 1987), the insulin VNTR was found to be associated with T1D (Haskins & Wegman 1996). Susceptibility in the *INS* region, or the *IDDM2* locus, was initially associated to a variable number of tandem repeats (VNTR) located ~0.5 kb upstream of (*INS*) (Vardi et al 1987, Haskins & Wegman 1996, Yu et al 2000). Homozygosity for the short class VNTR I alleles is found in ~75-85% of the patients compared to a frequency of 50-60% in the general population, suggesting that it predisposes to T1D. In contrast, homozygosity for the longer class III VNTR alleles is rarely seen in patients and the class III VNTR is believed to confer a dominant protective effect (Haskins & Wegman 1996, Pugliese et al 2001). The relative risk ratio of the I/I genotype vs. I/III or III/III has been reported to be moderate (in the 3-5 range) and it accounts for about 10% of the familial clustering of T1D (Jahromi & Eisenbarth 2006). However, analyses suggest that it is not possible to discriminate effects of the VNTR from other polymorphisms in this

region (Shaltout et al 2002) and that at least two other polymorphisms (-23HphI and +1140A/C) may be important (Heath et al 1998). Moreover, by measuring the *HphI* polymorphism (in tight linkage disequilibrium with the VNTR) (Stead et al 2000), Metcalfe and coworkers showed that homozygosity for the predisposing *INS* genotype increases the likelihood that identical twins will be concordant for T1D (Metcalfe et al 2001).

Insulin associated susceptibility and resistance may derive from quantitative differences in *INS* transcription in the specialized antigen presenting cells found in thymus and peripheral lymphoid tissues, where production of self-antigens such as proinsulin may be crucial for the shaping and maintaining of a self-tolerant T cell repertoire (Vafiadis et al 1997, Alizadeh & Koelman 2008). Such mechanisms may influence the probability of developing autoimmune responses to insulin as a key autoantigen in T1D.

### 2.2.2B2 LYP gene - PTPN22

LYP gene (encoded by PTPN22 gene belongs to a family of protein tyrosine phosphatases (PTPs) that are involved in modulating T-cell activation. The PTPN22 gene has been associated with development of T1D and other autoimmune diseases (Jahromi & Eisenbarth 2006). Recently, significantly associated of PTPN22 C1858T variant was found to be to lower fasting C-peptide levels, poorer glycemic control in recent onset of T1D subjects and to higher GADA in T1D patients with long disease duration (Mortensen et al 2010).

The gene encodes a lymphoid tyrosine phosphatase (LYP) which by dephosphorylation of Src family kinases negatively regulates T cell receptor (TCR) signaling. The current working hypothesis suggest that the risk carrying allele, T1858, suppresses TCR signaling more efficiently during thymic development resulting in survival of auto reactive T-cells (et al 2011). Bottini and co-workers evaluated a functional polymorphism in the *lyp* gene (no relation to the lymphopenia gene of the BB rat) in two series of patients with T1D, (Bottini et al 2004). They suggested the possible use of PTPN22 SNPs as a prognostic factor for disease severity and variability in autoimmune diseases (Bottini et al 2006).

There are evidence indicating that the diabetes associated allele may result in a “gain of function” (Bottini et al 2006) and thereby contribute to T cell activation. The odds ratio was approximately 1.7, making this polymorphism the most potent after HLA and *INS*. The polymorphism appears to be a missense mutation that changes an arginine at position 620 to a tryptophan and thereby abrogates the ability of the molecule to bind to the signaling molecule Csk (Bottini et al 2006, Begovich et al 2004). Consistent with a general effect on immune function is the finding that the minor tryptophan encoded allele is associated with a series of autoimmune disorders including T1D, and other autoimmune diseases (Begovich et al 2004, Kyogoku et al 2004). Multiple recent studies have confirmed the association of this missense mutation with T1D including an extensive study from United Kingdom. It is possible that polymorphisms in linkage disequilibrium with R620W determine increased risk of autoimmunity rather than the polymorphism, but this seems unlikely given the rapid confirmation of this polymorphism's association with multiple forms of autoimmune disease in multiple populations and its functional significance.

Recently Nielsen and coworkers have further investigated in the association of PTPN22 C1858T with the disease progression as assessed by liquid meal associated C-peptide and proinsulin, HbA1c, and daily insulin dose, IDAA1C (Petrone et al 2008), production of pancreatic islet antibodies and new onset of T1D (Mortensen et al 2009). They have shown that PTPN22 gene variant may be associated with changes in residual  $\beta$ -cell function and disease pathogenesis during the first year after onset of T1D (Nielsen et al 2011).



### 2.2.2B3 Cytotoxic T Lymphocyte Antigen-4

CTLA-4 (cytotoxic T lymphocyte-associated antigen 4) is a molecule on T-cells that plays a critical role in regulating natural immune responses. CTLA4 has attracted interest for many years, and multiple studies have established association or linkage between this chromosomal region and autoimmune diseases, particularly T1D (Nistico et al 1996, Ueda et al 2003, Anjos et al 2004, Concannon et al 2005). Cytotoxic T lymphocyte antigen-4 (CTLA-4) gene is located on chromosome 2q33, is one of the confirmed T1D susceptibility loci. This 300-kilobase region is known to contain at least three genes: CD28, CTLA-4, and the inducible costimulatory molecule (ICOS) gene (Kavvoura & Ioannidis 2005). The LD patterns in this region define two blocks, one comprising the CD28 gene and another including CTLA4 and the 5' end of ICOS. The first studies limited the signals to the CTLA4-ICOS block and subsequent research determined that the SNPs selected had functional effects on the CTLA-4 protein while the expression and function of ICOS did not suffer any change. The CTLA4 gene has four exons and three introns. Exon 1 codes for the leader peptide of the protein, exon 2 delivers the ligand-binding domain, exon 3 is the transmembrane domain and exon 4 the cytoplasmic tail. Two of the most studied and replicated polymorphisms in *CTLA4* are rs231775 (+A49G), located in exon 1, and rs3087243 (+6230G>A, also known as CT60) in the 3' region. The A allele of rs231775 codes for a threonine in position 17 of CTLA-4, forming a threonine-X-asparagin glycosylation site. The mutant G allele (alanine) causes an aberrant glycosylation of the derived protein and lower levels of membrane-bound CTLA-4 in *in vitro* experiments (Ueda et al 2003). The change CT60, a transition from a guanine to an adenine in position +6230 of the gene, is correlated to higher levels of a soluble isoform of the CTLA-4 protein.

The CTLA-4 receptor has two variants derived from alternative splicing: the membrane-bound and the soluble form that lacks the transmembrane domain. It is believed that the soluble isoform contributes to downregulate the activation of T cells by binding to CD80-CD86 receptors in antigen presenting cells and preventing the stimulation of CD28. Ueda and coworkers found a correlation between high levels of sCTLA-4 in serum and the protective A allele in the CT60 polymorphism (Ueda et al 2003). By mechanisms as yet unknown, the protective allele augments the levels of sCTLA-4 mRNA and patients who carry this allele have higher levels of free sCTLA-4 in serum, that most likely contribute to control the activation of the immune system.

Kavvoura and Ioannidis have a meta analysis on three of them and concluded that the A (49) G gene polymorphism in exon 1 is associated with T1D (Kavvoura & Ioannidis 2005). They have also reported a significant ethnic variation in that polymorphism. On the other hand, the A (CT60) G gene polymorphism have been studied in parallel with A (49) G in different in Caucasian as well as Asian population.

### 2.2.2B4 Other non-MHC genes and loci

Increasing studies have identified different non-MHC genes apart from insulin gene region, PTPN22, CTLA-4 to be associated in T1D pathogenesis. Other non-MHC genes might also be important in triggering the pathogenesis of T1D. However, further large scale confirmatory association studies might be required. The polygenic nature of T1D pathogenesis functions as a network of genes and loci that exert its effect in the triggering of the disease onset. Our series of human genetic of cytokines work have identified the effect of genetic polymorphisms in cytokines in the pathogenesis of T1D, Figure 1. Given the mediatory role of cytokines in the immune system they might be of a good therapeutic value. However, these findings require large scale confirmatory studies.

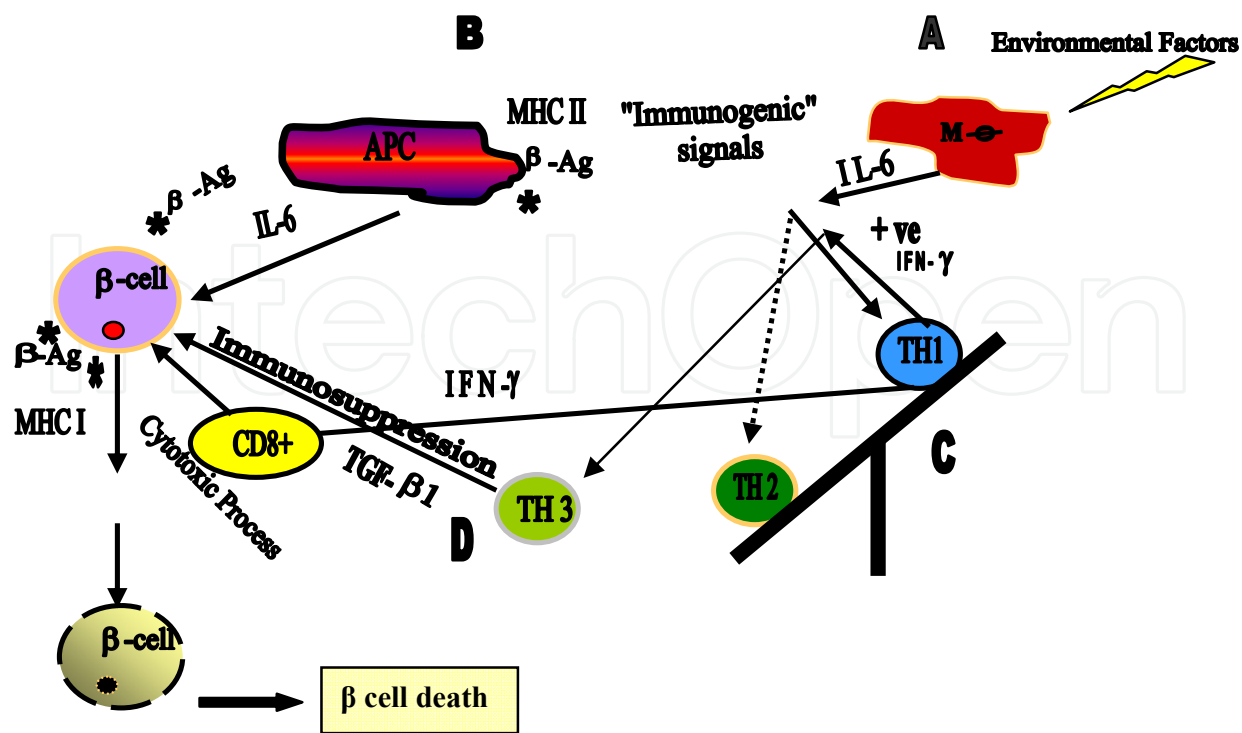


Fig. 1. Diagrammatic presentation of effects of cytokines gene polymorphisms in T1D. This schematic diagram explains the possible effects of cytokines in the initiation triggering of T1D by signals from either environmental factors “A” or immunogenic factors “B” or both. Release of IL-6 would change the counterbalance between TH1/TH2 “C” cytokines level. Increase level of TH1, IFN- $\gamma$ , cause the cytotoxic process of break down of  $\beta$ -cell by CD8. As a result of deviation of cytokine cross regulation to TH1 dominance TH3 lymphocyte subset produce immunosuppressive cytokines, TGF-  $\beta$ 1, however, due to genetic malfunction this cytokines are proved not to be able to bring TH1/TH2 back to normal position, D. Which will lead to the death of pancreatic  $\beta$  cell. This is a simplified schematic descriptions of the impact of cytokines in the pathogenesis of T1D, for detail please refer to Jahromi et al 2000, 2000<sup>a</sup> and 2010.

3. Conclusion

An increasing number of studies indicate that genetic factors influence both susceptibility to and resistance to T1D. Several chromosomal regions have been linked with the disease, suggesting the polygenic nature of disorder in most families. A few rare families have dramatic Mendelian mutations leading to immune mediated diabetes. Although DR and DQ alleles and their linkage disequilibrium pattern can explain a major portion of disease risk there is evidence for additional genes linked or within the MHC influencing risk. Combination of genes linked to the MHC, have a major effect on risk of T1D in addition to the known potent effects of HLA DR and DQ alleles. Further, other non-MHC genes such as INS, PTPN22 and CTLA-4 and others with small but significant effects might contribute to pathogenesis of T1D. Differences in disease risk between populations are likely due in part to the distribution within populations of DR and DQ haplotypes. However, the major genetic factors, probably linked to the MHC remain to be defined that will contribute to the remarkable differences in T1D incidence. With the

recent biotechnological advancements extensive genetic typing and the formation of cooperative global genetic study groups, international HapMap and Genome Wide Association (GWA) should enhance the speed at which true gene(s) for T1D will be unraveled.

#### 4. References

- Abdul Jabbar Al Abbassy, iman Farid and Dunia Al Hashimi 2002. HLA association with diabetes mellitus type 1 in Bahraini children. *Emirates med J*; 20: 143-146.
- Abdullah , M.A. 2005. Epidemiology of type I diabetes mellitus among Arab children Saudi. *Med J*. 26: 911-917.
- Alizadeh BZ, Koeleman BP 2008. Genetic polymorphisms in susceptibility to Type 1 Diabetes. *Clin Chim Acta*; 387:9-17.
- Alper, C.A., Z.Awdeh & E.J.Yunis 1992. Conserved, extended MHC haplotypes *Exp. Clin. Immunogenet.* 9: 58-71.
- Aly TA, Baschal EE, Jahromi MM, Fernando MS, Babu SR, Fingerlin TE, Kretowski A, Erlich HA, Fain PR, Rewers MJ, Eisenbarth GS 2008. Analysis of single nucleotide polymorphisms identifies major type 1A diabetes locus telomeric of the major histocompatibility complex. *Diabetes*;57:770-776.
- Aly TA, Ide A, Jahromi MM, Barker J M , Fernando MS, Babu SR, Yu L, Miao D, Erlich HA, Fain PR, Barriga KJ, Norris JM, Rewers MJ, Eisenbarth GS 2006. Extreme Genetic Risk for Type 1A Diabetes. *Proc Natl Acad Sci U S A* 103: 14074-14079.
- Anjos SM, Tessier MC, Polychronakos C 2004. Association of the cytotoxic T lymphocyte-associated antigen 4 gene with type 1 diabetes: Evidence for independent effects of two polymorphisms on the same haplotype block. *J Clin Endocrinol Metab*;89:6257-6265.
- Awata, T., T.Kuzua, A.Matsuda, Y.Iwamoto, Y.Kanzawa, M.Okuyama & T.Juji. 1990. High frequency of aspartic acid at position 57 of HLA-DQ B-chain in Japanese IDDM patients and nondiabetic subjects. *Diabetes* 39: 266-269.
- Barnett, A.H., C.EFF, R.D.Leslie & D.A.Pyke 1981. Diabetes in identical twins. A study of 200 pairs *Diabetologia* 20: 87-93.
- Begovich, A.B., V.E.Carlton, L.A.Honigberg, S.J.Schrodi, A.P.Chokkalingam, H.C.Alexander, K.G.Ardlie, Q.Huang, A.M.Smith, J.M.Spoerke, M.T.Conn, M.Chang, S.Y.Chang, R.K.Saiki, J.J.Catanese, D.U.Leong, V.E.Garcia, L.B.Mcallister, D.A.Jeffery, A.T.Lee, F.Batliwalla, E.Remmers, L.A.Criswell, M.F.Seldin, D.L.Kastner, C.I.Amos, J.J.Sninsky & P.K.Gregersen 2004. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis *Am. J. Hum. Genet.* 75: 330-337.
- Bodmer, JG; Marsh, SG; Albert, ED; Bodmer, WF; Dupont, B; Erlich, HA; Mach, B; Mayr, WR et al. (1992). "Nomenclature for factors of the HLA system, 1991". *Human immunology* 34 (1): 4-18.
- Bottini N, Vang T, Cucca F, Mustelin T 2006: Role of PTPN22 in type 1 diabetes and other autoimmune diseases. *Semin Immunol* ; 18:207-213.
- Bottini, N., Muscumecl L, A.Alonso, S.Rahmouni, K.Nika, M.Rostakhani, J.McMurry, G.Meloni, P.Lucarelli, M.Pellchia, G.Eisenbarth, D.Comings & T.Mustelin 2004. A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes *Nature Genet.* 36: 337-338.

- C. Brorsson, N. T. Hansen, K. Lage, R. Bergholdt, S. Brunak, F. Pociot, and Type 1 Diabetes Genetics Consortium 2009. Identification of T1D susceptibility genes within the MHC region by combining protein interaction networks and SNP genotyping data. *Diabetes Obes Metab.*;11:60-66.
- Caillat-Zucman, S., I.D Jilali-Saiah, J.Timsit, E.Bonifacio, V.Sepe, M.Shattack, G.F.Bottazzo, N.K.McLaren, M.Delamaire, S.Martin, A.-M.Yamamoto, TW.Meade, A.M.Valdes, M.C.Babron, E.Thorsby, F.Clerget-Darpoux, G.Thomson, J.F.Bach & The Participating Centers 1997. Insulin dependent diabetes mellitus (IDDM) joint report. 12th International Histocompatibility Workshop Study. In Genetic diversity of HLA. Functional and medical implications. Charron, D., Ed. 389-398. EDK. Paris.
- Concannon P, Erlich HA, Julier C, Morahan G, Nerup J, Pociot F, et al 2005. Type 1 diabetes: evidence for susceptibility loci from four genome-wide linkage scans in 1, 435 multiplex families. *Diabetes*;54:2995-3001.
- Escribano-De-Diego, J., P.Sanchez-Velasco, C.Luzuraga, J.G.Ocego-Vinyals, J.E.Paz-Mlguel & F.Leyva-Cobian 1999. HLA class II immunogenetics and incidence of insulin-dependent diabetes mellitus in the population of Cantabria (Northern Spain) *Hum. Immunol.* 60: 990-1000.
- Fain, P. & G.S.Eisenbarth 2002. Genetics of type 1 diabetes mellitus. In *Genetics in Endocrinology*. Baxter, J., S.Melmed, M.New & L.Martini, Eds. 37-72. Lippincott Williams & Williams. Philadelphia.
- Gianani R, Putnam A, Still T, Yu L, Miao D, Gill RG, Beilke J, Supon P, Valentine A, Iveson A, Dunn S, Eisenbarth GS, Hutton J, Gottlieb P, Wiseman A 2006. Initial results of screening of non - diabetic organ donors for expression of islet autoantibodies. *J Clin Endocrinol Metab* 91:1855-1861.
- Meier JJ, Bhushan A, Butler AE, Rizza RA, Butler PC 2005 Sustained beta cell apoptosis in patients with long-standing type 1 diabetes: indirect evidence for islet regeneration? *Diabetologia* 48:2221-2228.
- Haskins, K. & D.Wegmann 1996. Diabetogenic T-cell clones *Diabetes* 45: 1299-1305.
- Heath, V.L., N.C.Moore, S.M.Parnell & D.W.Mason 1998. Intrathymic expression of genes involved in organ specific autoimmune disease *J Autoimmun* 11: 309-318.
- Henry Erlich, Ana Maria Valdes, Janelle Noble, Joyce A. Carlson, Mike Varney, Hoffenberg EJ, Emery LM, Barriga KJ Bao, F, Taylor, J, Eisenbarth GS, Haas JE, Sokol RJ, Taki I, Norris J Cleary P Waberski, B, Greene DA, Stevens MJ, Feldman ELM, Rewers M 2004. Clinical features of children with screening-identified evidence of celiac disease. *Peds* 113:1254-1259.
- Homann D, Eisenbarth GS 2006. An immunologic homunculus for type 1 diabetes. *Journal of Clinical Investigation* 116: 1212-1215.
- Jahromi M, Millward A, Demaine A 2000 . A CA repeat polymorphism of the IFN-gamma gene is associated with susceptibility to type 1 diabetes. *J Interferon Cytokine Res.*; 20:187-190.
- Jahromi MM, Millward BA, Demaine AG 2000<sup>a</sup> . A polymorphism in the promoter region of the gene for interleukin-6 is associated with susceptibility to type 1 diabetes mellitus. *Interferon Cytokine Res.*;20:885-888.
- Jahromi MM, Millward BA, Demaine AG 2010. Significant correlation between association of polymorphism in codon 10 of transforming growth factor-beta1 T (29) C with type 1 diabetes and patients with nephropathy disorder. *J Interferon Cytokine Res*;30: 59-66.

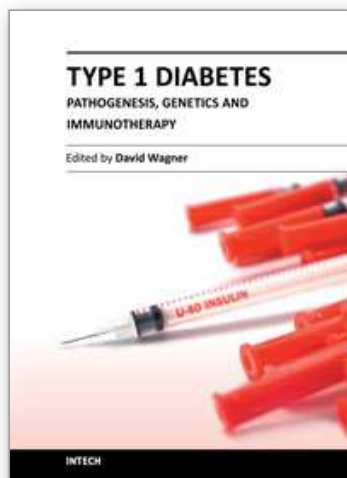


- Jeffrey C. Barrett, David Clayton, Patrick Concannon, Beena Akolkar, Jason D. Jeffrey C. Barrett, David Clayton, Patrick Concannon, Beena Akolkar, Jason D. Cooper, Henry A. Erlich, Cécile Julier, Grant Morahan, Jørn Nerup, Concepcion Nierras, Vincent Plagnol, Flemming Pociot, Helen Schuilenburg, Deborah J. Smyth, Helen Stevens, John A. Todd, Neil M. Walker, Stephen S. Rich, and the Type 1 Diabetes Genetics Consortium 2009. Genome-wide association study and meta-analysis finds over 40 loci affect risk of type 1 diabetes *Nat Genet.* ; 41(6): 703–707.
- Karl A Metcalfe, Graham A Hitman, Rachel R Rowe, Mohammed Hawa, Xiaojian Huang, Timothy Stewart, R David G Leslie. Concordance for Type 1 Diabetes in Identical twins is affected by Insulin genotype. *Diabetes Care* 2001; 24: 838-842.
- Karvoen , M., M.Viik-Kajander, E.Moltchanova, I.Libman, R.Laporte & J.TUomilehto 2000. Incidence of childhood type 1 diabetes worldwide. *Diabetes Mondiale (DiaMond) Project Group Diabetes Care* 23: 1516-1526.
- Kavvoura, F.K. & J.P.Ioannidis 2005. CTLA-4 gene polymorphisms and susceptibility to type 1 diabetes mellitus: a HUGE Review and meta-analysis *Am. J. Epidemiol.* 162: 3-16.
- Kawasaki, E. & K.Eguchi 2004. Is Type 1 diabetes in the Japanese population the same as among Caucasians? *Ann. N. Y. Acad. Sci.* 1037:96-103.: 96-103.
- Krishnamurthy B, Dudek NL, McKenzie MD Purcell AW, Brooks AG, Gellert S, Colman PG, Harrison LC, Lew AM, Thomas HE, Kay TW 2006. Responses against islet antigens in NOD mice are prevented by tolerance to proinsulin but not IGRP. *J Clin Invest* 116:3258-3265.
- Kyogoku, C., C.D.Langefeld, W.A.Ortmann, A.Lee, S.Selby, V.E.Carlton, M.Chang, P.Ramos, E.C.Baechler, F.M.Batliwalla, J.Novitzke, A.H.Williams, C.Gillett, P.Rodine, R.R.Graham, K.G.Ardlie, P.M.Gaffney, K.L.Moser, M.Petri, A.B.Begovich, P.K.Gregersen & T.W.Behrens 2004. Genetic Association of the R620W Polymorphism of Protein Tyrosine Phosphatase PTPN22 with Human SLE *Am. J Hum. Genet.* 75: 504-507.
- Liu E, Li M, Bao F Miao D, Rewers MJ, Eisenbarth GS, Hoffenberg EJ 2005. Need for quantitative assessment of transglutaminase autoantibodies for celiac disease in screening-identified children. *J Pediatr* 146: 494-499.
- Lotte B Nielsen, Sven Pörksen, Marie Louise M Andersen, Siri Fredheim, Jannet Svensson, Philip Hougaard, Maurizio Vanelli, Jan Åman, Henrik B Mortensen, Lars Hansen and the Hvidoere Study Group on Childhood Diabetes 2011. The PTPN22 C1858T gene variant is associated with proinsulin in new-onset type 1 diabetes. *BMC Med Genetics*; 12: 1-6.
- Marsh SG, Albert ED, Bodmer WF, Bontrop RE, Dupont B, Erlich HA, Geraghty DE, Hansen JA, Hurley CK, Mach B, Mayr WR, Parham P, Petersdorf EW, Sasazuki T, Schreuder GM, Strominger JL, Svejgaard A, Terasaki PI, and Trowsdale J 2005. "Nomenclature for factors of the HLA System, 2004". *Tissue Antigens* 65 (4): 301–369.
- Meier JJ, Bhushan A, Butler AE, Rizza RA, Butler PC 2005. Sustained beta cell apoptosis in patients with long-standing type 1 diabetes: indirect evidence for islet regeneration? *Diabetologia* 48:2221-2228.
- Mijovic, C.H., A.H.Barnett & J.A.Todd 1991. Genetics of diabetes. Trans-racial gene mapping studies. [Review] *Baillieres. Clin. Endocrinol. Metab.* 5: 321-340.



- Millward, B.A., L.Alviggi, P.J.Hoskins, C.Johnston, D.Heaton, G.F.Bottazzo, D.Vergani , R.D.Leslie & D.A.Pyke 1986. Immune changes associated with insulin dependent diabetes may remit without causing the disease: a study in identical twins *BMJ* 292: 793-796.
- Mitchell, Richard Sheppard; Kumar, Vinay; Abbas, Abul K.; Fausto, Nelson (2007). Robbins Basic Pathology. Philadelphia: Saunders. ISBN 1-4160-2973-7.8th edition.
- Mohamed M Jahromi and George S Eisenbarth. Cellular and Molecular Pathology of Type 1 Diabetes. *Cell Mol Life Sci* 2007; 64: 865-72.
- Mohamed M Jahromi and George S Eisenbarth. Genetic Determinants of Type 1 Diabetes Across Populations. *Ann NY Acad Sci* 2006; 289-299.
- Jahromi Mohamed M. Haplotype Specific Alteration of Type 1 Diabetes MHC Risk. (Submitted to *Diabetes Care*)
- Mortensen HB, Hougaard P, Swift P, Hansen L, Holl RW, Hoey H, Bjoerndalen H, de Beaufort C, Chiarelli F, Danne T, Schoenle EJ, Aman J, on behalf of the Hvidoere Study Group on Childhood Diabetes: New Definition for the Partial Remission Period in Children and Adolescents with T1D. *Diabetes Care* 2009, 32:1384-1390.
- Mortensen HB, Swift PG, Holl RW, Hougaard P, Hansen L, Bjoerndalen H, de Beaufort CE, Knip M, Hvidoere Study Group on Childhood Diabetes: Multinational study in children and adolescents with newly diagnosed type 1 diabetes: association of age, ketoacidosis, HLA status, and autoantibodies on residual beta-cell function and glycemic control 12 months after diagnosis. *Pediatr Diabetes* 2010, 11:218-226.
- Nakayama M, Abiru N, Moriyama H Babaya N, Liu E, Miao D, Yu L, Wegmann DR, Hutton JC, Elliott JF, Eisenbarth, GS 2005 Prime role for an insulin epitope in the development of type 1 diabetes in NOD mice. *Nature* 435:220-223.
- Nistico L, Buzzetti R, Pritchard LE, Van der Auwera B, Giovannini C, Bosi E, et al. The CTLA-4 gene region of chromosome 2q33 is linked to, and associated with, type 1 diabetes. *Belgian Diabetes*
- Pat Concannon, Josyf C. Mychaleckyj, John A. Todd, Persia Bonella, Anna Lisa Fear, Eva Lavant, Anthony Louey, and Priscilla Moonsamy for the Type 1 Diabetes Genetics Consortium. HLA DR-DQ Haplotypes and Genotypes and Type 1 Diabetes Risk:Analysis of the Type 1 Diabetes Genetics Consortium Families. *Diabetes* 2008; 57:1084-1092.
- Petrone, A., T.L.Bugawan, C.A.Mesturino, L.Nistco, A.Galgani, G.Giorgi, I.Cascino, H.A.Erich, U.Di Mario & R.Buzzetti 2001. The distribution of HLA class II susceptible/protective haplotypes could partially explain the low incidence of type 1 diabetes in continental Italy (Lazio region) *Tissue Antigens* 58: 385-394.
- Pugliese, A., D.Brown, Garza D, M.Zeller, M.J.Redondo, G.S.Eisenbarth, D.D.Patel & C.Ricodi 2001. Self-Antigen Presenting Cells Expressing Islet Cell Molecules in Human Thymus and Peripheral Lymphoid Organs: Phenotypic Characterization and Implications for Immunological Tolerance and Type 1 Diabetes *J Clin Invest* 107: 555-564.
- Redondo MJ, Fain PR, Eisenbarth GS. Genetics of type 1A diabetes.Recent Prog Horm Res 2001;56:69-89.
- Registry. *Hum Mol Genet* 1996;5:1075-1080.
- Shaltout AA, Moussa MA, Qabazard M, et al 2002 Further evidence for the rising incidence of childhood Type 1 diabetes in Kuwait. *Diabet Med*; 19:522-525.

- Souza F, Simpson N, Raffo A, Saxena C, Maffei A, Hardy M, Kilbourn M, Goland R, Leibel R, Mann JJ, Van Heertum R, Harris PE 2006 Longitudinal noninvasive PET-based beta cell mass estimates in a spontaneous diabetes rat model. *J Clin Invest* 116:1506-1513.
- Stead, J.D., J.Buard, J.A.Todd & A.J.Jefferys 2000. Influence of allele lineage on the role of the insulin minisatellite in susceptibility to type 1 diabetes *Hum. Mol. Genet.* 9: 2929-2935.
- Stene LC, Barriga K, Hoffman M, Kean J, Klingensmith G, Norris JM, Erlich HA, Eisenbarth GS, Rewers M. 2006 Normal but increasing hemoglobin A1c levels predict progression from islet autoimmunity to overt type 1 diabetes: Diabetes Autoimmunity Study in the Young (DAISY). *Pediatr Diabetes* 7:247-253.
- Todd, J.A., C.Mijovic, J.Fletcher, D.Jenkins, A.R.Bradwell & A.H.Barnett 1989. Identification of susceptibility loci for insulin-dependent diabetes mellitus by trans-racial gene mapping *Nature* 338: 587-589.
- Ueda H, Howson JM, Esposito L, Heward J, Snook H, Chamberlain G, et al. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature* 2003;423:506-511.
- Vafiadis P, Bennett ST, Todd JA, Nadeau J, Grabs R, Goodyer CG, et al. Insulin expression in human thymus is modulated by INS VNTR alleles at the IDDM2 locus. *Nat Genet* 1997;15:289-292.
- Vardi, P., A.G.Ziegler, J.H.Matthews, S.Dib, R.J.Keller, A.T.Ricker, J.I.Wolfsdof, R.D.Herskowitz, A.Rabizadeh, G.S.Eisenbarth & J.S.Soeldner 1988. Concentration of insulin autoantibodies at onset of type I diabetes. Inverse log-linear correlation with age *Diabetes Care* 11: 736-739.
- Vardi, P., S.A.Dib, M.Tuttleman, J.E.Connelly, M.Grinbergs, A.Rabizadeh, W.J.Rilley, N.K.McLaren, G.S.Eisenbarth & J.S.Soeldner 1987. Competitive insulin autoantibody assay. Prospective evaluation of subjects at high risk for development of type I diabetes mellitus *Diabetes* 36: 1286-1291.
- Yu, L., D.T.Robles, N.Abiru, P.Kaur, M.Rewers, K.Kelman & G.S.Eisenbarth 2000. Early expression of antiinsulin autoantibodies of humans and the NOD mouse: evidence for early determination of subsequent diabetes *Proc Natl Acad Sci USA* 97: 1701-1706.
- Zhang Xiao-mei, Wang Hong-yuan, Luo Ying-ying and Ji Li-nong. HLA-DQ, DR allele polymorphism of type 1 diabetes in the Chinese population: a meta-analysis. *Chin Med J* 2009;122(8):980-986.



## **Type 1 Diabetes - Pathogenesis, Genetics and Immunotherapy**

Edited by Prof. David Wagner

ISBN 978-953-307-362-0

Hard cover, 660 pages

**Publisher** InTech

**Published online** 25, November, 2011

**Published in print edition** November, 2011

This book is a compilation of reviews about the pathogenesis of Type 1 Diabetes. T1D is a classic autoimmune disease. Genetic factors are clearly determinant but cannot explain the rapid, even overwhelming expanse of this disease. Understanding etiology and pathogenesis of this disease is essential. A number of experts in the field have covered a range of topics for consideration that are applicable to researcher and clinician alike. This book provides apt descriptions of cutting edge technologies and applications in the ever going search for treatments and cure for diabetes. Areas including T cell development, innate immune responses, imaging of pancreata, potential viral initiators, etc. are considered.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Mohamed M. Jahromi (2011). Genetic Determinants of Type 1 Diabetes, Type 1 Diabetes - Pathogenesis, Genetics and Immunotherapy, Prof. David Wagner (Ed.), ISBN: 978-953-307-362-0, InTech, Available from: <http://www.intechopen.com/books/type-1-diabetes-pathogenesis-genetics-and-immunotherapy/genetic-determinants-of-type-1-diabetes>

**INTech**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen