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**MINIREVIEW**

# Genetic Susceptibility to Type 1 Diabetes Mellitus in Humans

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## Summary

Type 1 diabetes mellitus (DM 1A) is an autoimmune disease belonging to the most frequent chronic diseases of the childhood and young adults. DM 1A results from immune-mediated destruction of the insulin-producing beta cells of the pancreas. It is a genetically determined disease and many genes or genetic regions were found to be associated with its induction. In addition to the insulin-dependent diabetes mellitus 1 (IDDM1) gene, which marks the HLA region, and IDDM2 which marks the insulin gene, significant associations of DM 1A to other IDMM genes or genetic regions we reported. We shortly review recent achievements in the field, and the state of current knowledge.

**Key words**

CD25 • CTLA4 • HLA • IDDM • Insulin • Type 1 diabetes mellitus • PTPN22

## Introduction

Type 1A diabetes mellitus (DM 1A; MIM 222100) is one of the most common chronic diseases of childhood and the most common type of diabetes in persons under 40 years of age. It is the leading cause of blindness, amputations, and end-stage renal disease, and contributes to premature death. The most frequent age of its onset is 12-13 years, but it may occur at any age, in all racial groups, with equal prevalence (about 1/300) in males and females. The incidence of type 1A diabetes has been increasing in many countries (Robles and Eisenbarth 2001, Gottlieb and Eisenbarth 2002).

Almost one half of monozygotic twins of patients with DM 1A develops diabetes. The concordance of monozygotic (50 %) and dizygotic (5 %) twins for DM 1A differs dramatically. The probability of a monozygotic twin living in different environmental conditions to develop to diabetes decreases with the duration of discordance, but twins can become concordant more than 40 years after the development of diabetes in their twin sibling. The risk for diabetes of a dizygotic twin is more or less similar to the risk of a twin of a patient with diabetes (5 %). Thus the shared environment of dizygotic twins does not appear substantially enhance the development of diabetes. Expression of anti-islet autoantibodies is much greater for monozygotic twins as compared to dizygotic twins. The majority of monozygotic twins of DM 1A patients expressing anti-islet autoantibodies progresses to diabetes

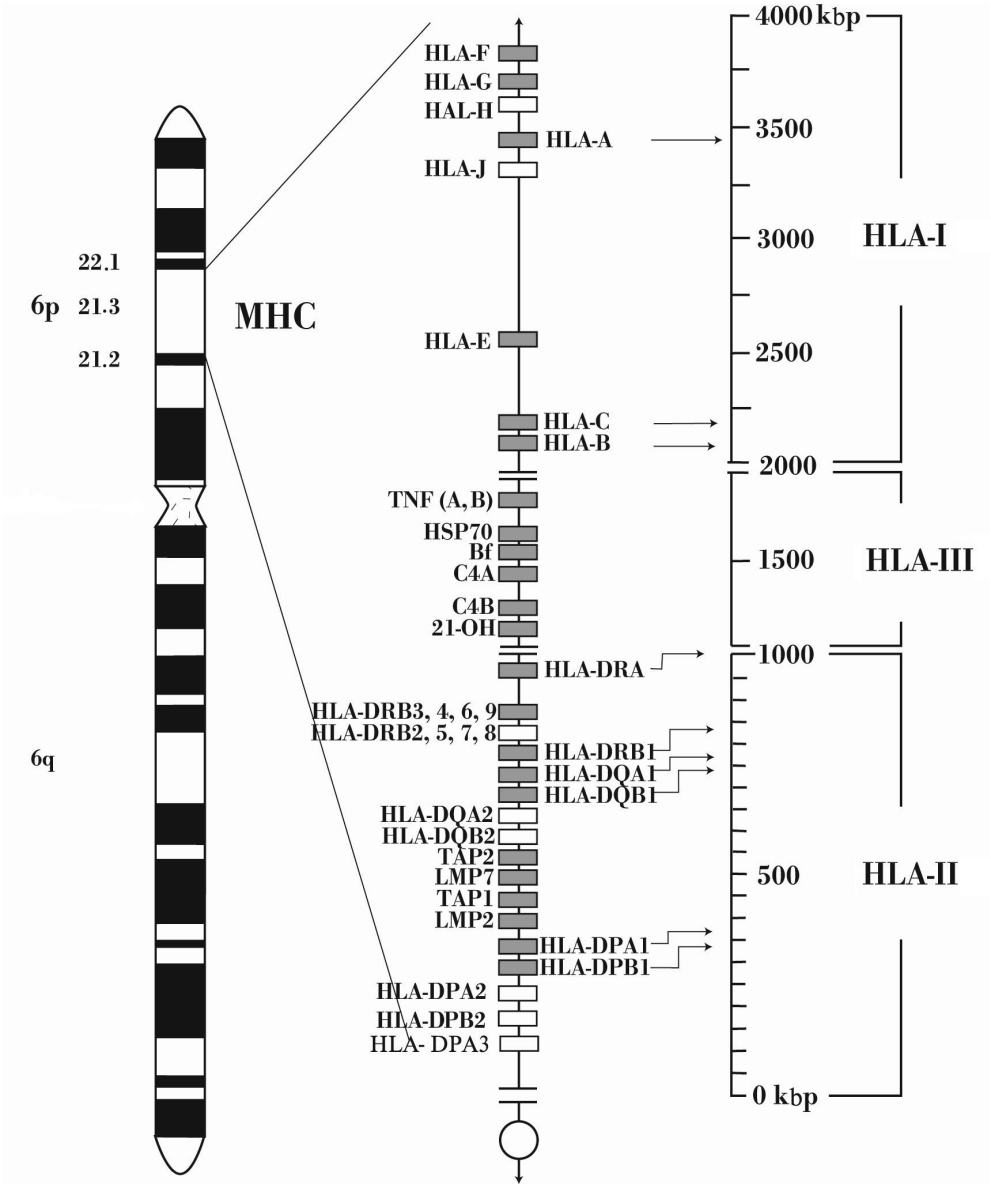


**Fig. 1.** Genetic region of the major histocompatibility complex in man. The genetic region of the major histocompatibility complex in man, HLA, is located on the short arm of chromosome 6 (6p21.3) and occupies a large segment of DNA, extending for about 3600 kbp. It is a region of highly polymorphic genes that form separate gene clusters, class I (telomeric) and class II (centromeric). These two regions are separated by another cluster of unrelated genes, which are called class III genes. The classical class I HLA loci are HLA-A, -B, and -C, those of class II are HLA-DR (DRA, DRB), -DQ (DQA, DQB) and -DP (DPA, DPB). Class III HLA region comprises the complement genes C2, C4, Bf, heat shock proteins genes (HSP70), tumour necrosis factor genes (TNF), 21-hydroxylase (21-OH) and others.

(Redondo *et al.* 1999, Gottlieb and Eisenbarth 2002).

DM 1A results from immune-mediated destruction of the insulin-producing beta cells of the pancreas. Autoimmune diabetes in the non-obese diabetic (NOD) mouse shares many genetic and pathophysiological characteristics with human DM 1A. In both species, the major histocompatibility complex (MHC) and multiple non-MHC genes contribute to disease susceptibility. However, the genes of the MHC confer the highest relative risks of disease development, between 3 to 50 for the most predisposing haplotypes and genotypes compared to less than 3 of non-MHC genes.

## HLA genes in predisposition to DM 1A

The genetic region of the major histocompatibility complex in man, HLA (human leukocyte antigens), is located on the short arm of chromosome 6 (6p21.3) and occupies a large segment of DNA, extending about 3600 kbp, i.e. in classical terms,

3.6 cM. It is a region of highly polymorphic genes that form separate gene clusters, class I (telomeric) and class II (centromeric). These two regions are separated by another cluster of unrelated genes called class III (for review see Buc 1993, 2005, Žalkovičová *et al.* 1998) (Fig. 1). A major determinant of genetic susceptibility resides in the HLA class II region. HLA class II molecules, particularly DR and DQ, account for approximately 40 % of the genetic risk for DM 1A development (Jones *et al.* 2006). As the HLA region displays a significant degree of linkage disequilibrium (i.e. specific DQ and DR alleles are non-randomly associated with each other), associations of HLA alleles with disease must be considered as haplotype specific and not allele specific. HLA class I alleles can also influence the disease, and it is possible that unknown genes linked to the HLA region are also important.

Individuals with the highest risk for type 1A diabetes express both predisposing haplotypes: DQA1*0501-DQBl*0201 (DQ2), which is almost always inherited with DRBl*0301 (DR3) and DQA1*0301-DQBl *0302 (DQ8), inherited with DRBl*0401 or DRBl*0402 (DR4) (Chowdhury *et al.* 1999, Nepom 2000, Černá *et al.* 2003, Buc *et al.* 2006). These individuals have been referred to as DR3/DR4 or DQ2/DQ8 heterozygotes. Individuals who carry this high risk haplotypic combination have ~5 % absolute risk of DM 1A. However, within affected families, this genotype has ~20 % risk; approximately 40 % of diabetic children have this genotype compared to 2 % of children in the healthy population (Chowdhury *et al.* 1999, Nepom 2000, Concannon *et al.* 2005). Observations of transmission frequencies of particular haplotypes have helped to illustrate the importance of certain haplotypes in contributing to diabetes susceptibility. For example, analysis of Human Biological Data Interchange (www.ncbi.nlm.nih.gov) family collection has revealed that DQA1*0501-DQB1*0201 and DQA1*0301-DQBl*0302 are transmitted to more than 80 % of diabetic children.

HLA-alleles have also been associated with protection from type 1A diabetes, especially the haplotype DQA1*0102/DQB1*0602/DRB1*1501 confers protection. Evidence suggests that such protection may mostly be encoded by the DQB1*0602 allele and even the first-degree relatives with islet cell antibodies (ICA) have a low diabetes risk if they carry DQB1*0602. However, this protective effect is not absolute (Chowdhury *et al.* 1999, Redondo *et al.* 2000, Sanjeevi 2000, Greenbaum *et al.* 2002). To summarize, as some HLA haplotypes are associated with high, moderate, low risks and even "protection", their identification is useful in disease prediction (Table 1).

**Table 1.** Spectrum of diabetes risk HLA haplotypes

| | | | |
|---|---|---|---|
| **High risk haplotypes** | | | |
| DR3 | DRB1*0301 | DQA1*0501 | DQB1*0201 |
| DR4 | DRB1*0401 | DQA1*0301 | DQB1*0302 |
| | DRB1*0402 | DQA1*0301 | DQB1*0302 |
| | DRB1*0405 | DQA1*0301 | DQB1*0302 |
| **Moderate risk haplotypes** | | | |
| DR1 | DRB1*01 | DQA1*0101 | DQB1*0501 |
| DR8 | DRB1*0801 | DQA1*0401 | DQB1*0402 |
| DR9 | DRB1*0901 | DQA1*0301 | DQB1*0303 |
| **Protective haplotypes** | | | |
| *Strong protection* | | | |
| DR2 | DRB1*1501 | DQA1*0102 | DQB1*0602 |
| DR6 | DRB1*1401 | DQA1*0101 | DQB1*0503 |
| DR7 | DRB1*0701 | DQA1*0201 | DQB1*0303 |
| *Moderate protection* | | | |
| DR5 | DRB1*1101 | DQA1-0501 | DQB1*0301 |
| *Weak protection* | | | |
| DR4 | DRBl*0401 | DQAl*0301 | DQBl*0301 |
| | DRBl*0403 | DQAl*0301 | DQB1*0302 |
| DR7 | DRB1*0701 | DQAl*0201 | DQBl*0201 |

There are also some reports on association between the last group of class II HLA alleles and DM 1A. HLA-DPB1*0101, DPB1*0301 and DPB1*0202 were reported to be positively and DPB1*0402 negatively associated (Noble *et al.* 2000, Valdes *et al.* 2001, Stuchlíková *et al.* 2006).

## The insulin gene

The second well established susceptibility locus in DM 1A is on chromosome 11p15.5, which is mapped to a region containing the variable number of tandem repeat (VNTR) polymorphism in the promoter region of the insulin gene. This VTNR region is categorized into classes I to III. VNTR I contains 26-63 repeating units

(5′- - - ACAGGGGTGGTGGGG - - - 3′), VNTR II 80 units, and VNTR III 140-210 units, respectively. Occurrence rate of VNTR I in the Caucasian population is approximately 70 %, that of VNTR III 30 %, and VNTRII occurs very rarely.

VNTR I homozygous individuals develop DM 1A more likely than those with VNTR III; VNTRII is associated with resistance to the disease induction (Bennet *et al.* 1995, Barrat *et al.* 2004, Maier and Wicker 2005, Královičová *et al.* 2006).

The thymic expression of insulin, and the deletion of autoreactive T cells during the process of negative selection, is under regulation of the transcription factor AIRE (autoimmune regulator) (Venazi *et al.* 2004, Villasenor *et al.* 2005, Buc, 2005). The type of the VNTR promoter region of the insulin gene may therefore influence the binding of AIRE transcription factor. Thus VNTR I allele predispose to DM 1A by reducing tolerance to insulin and its precursors *via* lower insulin transcription in thymic medullary epithelial cells. These reports are consistent with studies demonstrating that positivity for insulin autoantibodies has a high predictive value for DM 1A (Kukko *et al.* 2005).

HLA and insulin (INS) regions account for almost 60–70 % of the familial aggregation of type 1A diabetes. In some populations, the combined effects of HLA and INS contribute less than 50 % of the familial increased diabetes risk. Therefore, several genome-wide linkage studies have been conducted to identify candidate regions that may contain unidentified susceptibility genes. About 20 candidate regions for diabetes genes have been reported in linkage studies of affected sibling pairs (Concannon *et al.* 2005). Most of the known or suspected susceptibility loci have been designated IDDM, e.g. IDDM1 refers to genes mapping to the HLA region at 6p21, IDDM2 to the insulin region at 11pl5 etc. (Table 2). Although the statistical analysis varies across studies, none of the proposed susceptibility loci has an effect as strong as that associated with the HLA region on chromosome 6. An added complexity has been the inability to confirm certain loci in different population groups. This might occur if the identified regions are "false" positive associations or if different combinations of non-HLA genes contribute to autoimmune diabetes in different populations (Pociot and McDermott 2002, Concannon *et al.* 2005).

In disorders following a Mendelian pattern of autosomal dominant or recessive transmission, the pattern of inheritance of the disease phenotype is usually obvious. It is much more difficult to confidently define the reported linkages to diabetes susceptibility genes, since the mode of inheritance of the genes causing these complex disorders is unknown. There is considerable controversy regarding the interpretation of linkage studies for complex diseases. The LOD score has been used as a measure of the statistical evidence for linkage between a marker and a gene. In fact, the primary motivation for genome-wide linkage searches is to provide definitive evidence that one or more of such genes exist. "Lamda-s" values reflecting sibling risk of a disease in relation to its population prevalence are calculated, too. In DM 1A they are in the range 2.5 to 4.5 for HLA compared to 1.0-1.6 for other IDDM loci (Table 2).

## Cytotoxic T lymphocyte antigen-4 (CTLA-4)

The CTLA-4 gene is localized on the long arm of chromosome 2 (2q33) and this genetic region, IDDM12, was previously found to be associated with the predisposition to type 1 diabetes mellitus. Later it was proved that this association belongs to CTLA-4 gene (Kristiansen *et al.* 2000a, Ueda *et al.* 2003).

CTLA-4, a disulfide-linked homodimer expressed on the cell surface of activated T-cells is responsible for attenuation of the immune response by binding to ligands CD80 or CD86 expressed on the surface of antigen presenting cells. The CTLA-4 – CD80/CD86 interaction down-regulates the alpha chain of IL-2 receptor (CD25) expression what is followed by a decreased synthesis of IL-2 or may induce apoptosis in previously activated cells (Noel *et al.* 1996, Bucová 2002). CTLA-4 is also an important molecule by which $CD^+CD25^+$ T regulatory cells (Treg) exert their suppressive activity (Paust *et al.* 2004). They represent naturally occurring population of $CD4^+$ T cells that are vital in the control of autoimmune and inflammatory responses.

Because of the important role in regulation of the immune response, CTLA4 is a very strong candidate for association with autoimmune diseases. Indeed, the associations of CTLA-4 polymorphism with autoimmune diseases, such as Graves′s disease, autoimmune hepatitis were reported. As for DM 1A, important associations were also found. The association was found in the 3′ flanking region, downstream of the previously known polyadenylation site; it is a single nucleotide polymorphism (SNP): +6230G>A. This polymorphism may be associated with altered levels of steady state

**Table 2.** DM 1A susceptibility loci.

| Locus | Region | $\lambda_s$* | LOD | References |
|---|---|---|---|---|
| IDDM1 *(HLA)* | 6p21 | 1.7–4.2 | 65.8 | Cox *et al.* 2001 |
| IDDM2 *(INS)* | 11p15 | 1.6 | 4.28 | Davies *et al.* 1994, Cox *et al.* 2001 |
| IDDM3 | 15q26 | | | Zamani *et al.* 1996 |
| IDDM4 | 11q13 | 1.0–1.5 | 2.7 | Nakagawa *et al.* 1998 |
| IDDM5 | 6q25 | | 4.5 | Luo *et al.* 1996 |
| IDDM6 | 18q12-q21 | 1.0–1.5 | 1.1 | Laine *et al.* 2004 |
| IDDM7 | 2q31 | 1.0–1.6 | 1.2 | Copeman *et al.* 1995, Kristiansen *et al.* 2000b |
| IDDM8 | 6q27 | 1.0–2.1 | 3.6 | Luo *et al.* 1996 |
| IDDM9 | 3q22-q25 | 1.0–1.7 | 3.4 | Laine *et al.* 2006 |
| IDDM10 | 10p11-q11 | 1.1–2.2 | 2.8 | Cox *et al.* 2001, Mein *et al.* 1998 |
| IDDM11 | 14q24-q31 | | 4.0 | Field *et al.* 1996 |
| IDDM12 *(CTLA4)* | 2q31-q33 | | 3.57 | Marron *et al.* 1997, Cox *et al.* 2001 |
| IDDM13** | 2q34-q35 | | | Larsen *et al.* 1999 |
| IDDM15 | 6q21 | | 2.36 | Delepine *et al.* 1997, Cox *et al.* 2001 |
| IDDM16 *(IGH)* | 14q32 | | | Field *et al.* 2002 |
| IDDM17 | 10q25 | | 2.38 | Babu *et al.* 2004 |
| IDDM18 *(IL-12p40)* | 5q33 | | | Luo *et al.* 1996, Morahan *et al.* 2001 |
| | 7q25 | | 1.81 | Cox *et al.* 2001 |
| | 16q22-q24 | 1.6 | 3.93 | Cox *et al.* 2001 |
| | 1q42 | | 2.2 | Cox *et al.* 2001 |
| | 8q22-q24 | | 2.4 | Sale *et al.* 2002 |

IGH - immunoglobulin heavy chain, * Gottlieb and Eisenbarth 2002, **The symbol *IDDM14* has been reserved but not published.

mRNA of a soluble CTLA-4 through as yet unknown mechanisms (Marron *et al.* 1997, Ueda *et al.* 2003).

In addition to the effect of the 3'-flanking +6230G>A on splicing or RNA stability, there are also promoter polymorphisms that could contribute to the DM-1A association through transcriptional effects on expression. Anjos *et al.* (2004) found that the promoter polymorphism, -319C>T, is highly associated with the disease.

Another association between CLTA-4 and DM 1A is related to the position +49 of the CTLA-1 gene. The non-synonymous +49A>G base substitution is responsible for Thr17Ala change in the signal peptide. The predisposing Ala17 allele is incompletely glycosylated in the endoplasmic reticulum leading to retrograde transport of a portion of the molecules into the cytoplasm for degradation. This ultimately results in less CTLA-4 (Ala17) at the cell surface, which may explain the reduced inhibitory function of CTLA-4 reported in patients with +49G allele (Anjos *et al.* 2002, Mauer *et al.* 2002).

## PTPN22 is associated with autoimmune disease

The fourth established human DM 1A susceptibility locus is PTPN22 (protein tyrosine phosphatase non-receptor type 22; 1p13). It encodes a lymphoid protein tyrosine kinase (LYP) that is important on the negative control of T-cell activation and in T-cell development. A non-synonymous single nucleotide polymorphism at nucleotide 1858 in codon 620 (Arg620Trp) in PTPN22 was associated with type 1 diabetes (Smyth *et al.* 2004). The disease-associated variant Trp620 may alter the binding of LYP to the cytoplasmic tyrosine kinase (Bottini *et al.* 2004), which regulates the T-cell receptor-signaling kinases, T-cell-specific protein tyrosine kinase (LCK) and FYN, respectively (Hill *et al.* 2002), ultimately leading to hyperreactive T cell responses. Similar to CTLA4, PTPN22 is a DM 1A susceptibility locus that is shared by several organ-specific and systemic autoimmune diseases (rheumatoid arthritis, systemic lupus eruthematosus) (Maier and Wicker 2005).

## Association with the gene encoding the alpha chain of the IL-2 receptor

It is well recognized that interleukin-2 (IL-2) has paradoxical functions in T cell homeostasis, acting as a potent T cell growth factor during the initiation of immune responses and having a crucial function in the termination of T cell responses and maintenance of self-tolerance. The latter function has been proposed to be due to a requirement for IL-2 signaling for the development and function of regulatory T cells (Treg). Although IL-2 signaling is not required for their development in the thymus, it is critical for maintaining Treg cells in the peripheral T cell pool. Its levels might therefore affect disease susceptibility *via* the mechanisms that maintain immune homeostasis. It has been proved that although the levels of $CD4^+CD25^+$ regulatory T cells were normal in patients with DM 1A, their ability to suppress T-cell proliferation during *in vitro* co-cultures was markedly reduced compared with control subjects (Lindley *et al.* 2005, Maloy and Powrie 2005). Moreover, these co-cultures displayed a more pro-inflammatory phenotype, with increased secretion of IFN-γ and decreased IL-10 production. From this point of view, it is very interesting that the region containing the gene IL2RA encoding the alpha chain of the IL-2 receptor (CD25) on chromosome 10p15-p14 could be the fifth susceptibility locus for human DM 1A (Vella *et al.* 2005).

## Other IDDM loci involved in susceptibility to DM 1A

*IDDM4* is a region on chromosome 11q13 and one of its genes which might be involved in DM 1A genetic predisposition, can be that coding for FADD (Fas-associated death domain containing protein), a molecule involved in the apoptosis process (Nakagawa *et al.* 1998). *IDDM7* on chromosome 2q31-33 may be identical with NeuroD gene that is involved in morphogenesis of beta cells of Langerhans islets (Copeman *et al.* 1995, Esposito *et al.* 1998). NRAMP1 gene (natural resistance associated macrophage protein) is probably identical with *IDDM13*, a region on the long arm of chromosome 2 (2q34-35) (Esposito *et al.* 1998). It encodes a protein responsible for macrophage resistance to intracellular parasitic bacteria. NRAMP1 is also associated with a susceptibility to rheumatoid arthritis (Shaw *et al.* 1996), which indicates that more autoimmune disorders will share susceptibility genes. *IDDM18* (5q31.1-q33.1) is a genetic region harboring p40 chain of interleukin 12 (IL-12) (Bergholdt *et al.* 2004). IL-12 is a disulphide linked heterodimer composed of a heavy chain of Mr 40000, p40, and a light chain of Mr 35000, p35, encoded by their respective genes. The gene for the p35 subunit is located on chromosome 3p12-q13.2. The heterodimer is the biologically active IL-12. It drives the differentiation of T cells towards the TH1 subset, characterized by production of cytokines leading to cell mediated immunity. IL-12-induced autoreactive T cell responses might predispose to self destructive immunity. Finally, there is also a report on the association of *CD4 SNP promoter polymorphism* and type 1 diabetes mellitus (Kristiansen *et al.* 2004). CD4 is a principal differentiation antigen of T helper cells involved in cell cooperation and signal transduction.

A search for the genetic determination of type 2 diabetes mellitus (T2 DM non-insulin-dependent diabetes mellitus) has also been performed. Several studies have reported an increased frequency of T2 DM in families with type 1 diabetes (Dahlquist *et al.* 1989, Corel *et al.* 1993, Martinka *et al.* 1999, Li *et al.* 2001). On the other hand, frequent occurrence of DM 1A in relatives of patients with T2 DM has been observed (Quatraro *et al.* 1987, Gottlieb 1980). A parental history of T2 DM is associated with an increased risk of DM 1A in siblings of type 1 diabetic patients (Wagener *et al.* 1982). Thus, type 1 diabetes susceptibility genes could contribute to the polygenic etiology of type 2 diabetes and modify its clinical manifestation.

## Environmental factors

The annual incidence of type 1 diabetes mellitus amongst children varies dramatically from more than 40 per 100 000 children in Finland to less than 2 per 100 000 in Japan (Onkamo *et al.* 1999, Gottlieb and Eisenbarth 2002). In addition, there is compelling evidence of a temporal increase in the incidence of DM 1A, with countries such as Finland experiencing more than a doubling in incidence over the past four decades (Drykoningen *et al.* 1996, Gardner *et al.* 1997). How much of the variation in incidence between countries is due to environmental factors and how much is due to genetic differences between populations is unknown. For Sardinia, with one of the highest incidences of childhood diabetes in the world, the major factor appears to be genetics, as migrants from Sardinia to the Italian mainland have maintained their high incidence of type 1

diabetes (Muntoni *et al.* 1997).

The temporal increase in the incidence of DM 1A is almost certainly due to environmental factors. Moreover, it was noted that the incidence of diabetes had seasonal variation with an increase in children presenting with the disorder in the fall and winter suggesting viral infections might precipitate the disease. A series of virus candidates include picornaviruses, rotaviruses, herpesviruses, mumps, rubella, and retroviruses (Yoon *et al.* 1979, Dahlquist 1998, Gottlieb and Eisenbarth 2002). Coxsackie viruses have been of particular interest because of a homology between the virus and the target antigen glutamic acid decarboxylase 65 (GAD65); both negative and positive studies have been reported (Atkinson *et al.* 1994, Horwitz *et al.* 1998, Heino *et al.* 2001). A possible explanation for the lack of definitive association between viral infection and diabetes induction may derive from the fact that viral factors are ubiquitous and not unique to those with diabetes.

Nutritional factors were suggested to induce immunopathological processes, too. Antibodies to milk proteins and T cell responses to these proteins were reported to be increased among children with DM 1A. This includes a molecule ICA69 with some homology to bovine albumin (Stassi *et al.* 1997). Moreover, certain forms of milk casein vary in their protein sequence and might be converted to peptides cross-reactive to peptides derived from self-proteins (Elliot *et al.* 1999). A number of retrospective studies have implicated early ingestion of milk to the development of diabetes. The increased risk in most of these studies is, however, small and prospective studies following children till the expression of autoimmunity have failed to find an association between milk ingestion and anti-islet autoimmunity (Norris *et al.* 1996).

Overall, the search for environmental factors contributing to the development of diabetes has been relatively disappointing. With the exception of congenital rubella infection, none has been confirmed. Prospective studies will perhaps bring more light to the problem.

## Conclusions

The functions of gene products of so far established type I diabetes mellitus loci in humans and the NOD mouse suggest that a fine balance in T cell development, expansion, homeostasis and reactivity needs to be maintained to protect against autoimmune destruction of the pancreatic islets. The arrival of modern genotyping technologies and availability of large numbers of SNPs across the genome will make the discovery of many more genes possible. The identified susceptibility genes will aid in disease prediction and provide an insight into the underlying DM1A etiology and pathology, and possibly uncover as yet unexplored avenues leading to disease prevention and/or treatment.

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## Abbreviations

cM – centiMorgan
kbp – kilobase pairs
LOD – logarithm of the odds
MIM – Mendelian inheritance in man

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