Abstract
Type 1 diabetes (T1D) is a chronic immune-mediated disease, characterised by a selective loss of insulin-producing β-cells in the pancreatic islets. Susceptibility is determined by interactions of multiple genes with unknown environmental factors. Around 50% of the genetic risk of the disease is explained by HLA, although other genes with a smaller effect are also involved. Most of the known risk genes for T1D play a role in immunity, mostly through T-cell regulation (CTLA4, PTPN22, IL-2RA) and cytokine production or modulation (VDR, SUMO4). The insulin gene (INS) represents an exception to this, and is probably the only gene specifically associated with T1D and not with other autoimmune diseases. Ongoing genome-wide association studies are providing evidence of multiple known and previously unknown risk genes. New analytical tools are continuously being developed to handle the vast amounts of data produced, as well as to account for multiple comparisons and assess combined effects such as gene-gene and gene-environment interactions. In this review, we will give an overview of the most important genes identified to date, analyse the genetic evidence supporting them as T1D susceptibility genes and discuss the mechanisms mediating their contribution to the pathogenesis of the disease.

Keywords: autoimmune, insulin-dependent, family risk, prediction.

Introduction
Type 1 diabetes (T1D) is one of the most common chronic diseases in childhood, with an incidence ranging from 0.1 to 64/100,000 per year (China vs Finland) and increasing in most of the countries where it has been studied DIAMOND and EURODIAB.1,2,5 In Spain, according to most studies, the incidence per 100,000 inhabitants and year ranges between 9.5 and 16.4 for children diagnosed before the age of 15.6-13 An exception to these are represented by recent results from Castilla-Leon, showing an incidence of 22/100,000/yr,1 from Ciudad Real (26/100,000/yr)15 and data from the Canary Islands. The latter region has the highest rate of childhood T1D ever reported in Spain, with the most recent incidence (32/100,000/yr on the island of La Palma) approaching that of some Northern European countries.16,17 In addition, some studies show an increasing trend in the incidence of T1D in Spain,12,16 whereas others do not.6

Type 1A diabetes (T1D) is a complex, multifactorial disease, which leads to the autoimmune destruction of pancreatic, insulin-producing, β-cells. Its ultimate cause is unknown, but both environmental and genetic factors have proved to play a role in its development. The present review will focus on the latter. Although only 10-12% of patients with T1D have a family history of the disease at diagnosis, with longer follow-up the frequency increases to up to 25%.18 In addition, having a first degree relative with the disease increases the risk from 2.5 to more than 100 times, depending on who the affected family member is (table 1).

The most established genetic locus contributing to T1D and many other autoimmune diseases is the major histocompatibility complex (MHC), which includes the genes encoding the human leukocyte antigen gene (HLA), crucial in antigen presentation. In addition, several other genes have been established as contributing to the development of the disease, although to a smaller extent than HLA. More than 20 different loci have been proposed to account for the genetic risk of T1D (figure 1), but only a minority had been confirmed until very recently. At
present, we are witnessing an «explosion» of risk genes, thanks to the availability of new genotyping technologies and bioinformatic tools.

Recent development in genetic research
The genotyping and analytical tools made available in recent years have profoundly changed the way we understand genetics research. Genotyping methods have allowed for several hundreds of thousands genetic variations to be studied, as opposed to only a few at a time with previous technology. This fact has also changed the strategy of studies, moving from directed, hypothesis-based search for candidate genes to non hypothesis-based, unbiased whole genome association studies which complement the former. Sample sizes have increased from a few hundreds to many thousands of patients. In complex diseases like diabetes, this increase in sample size is crucial if we aim to identify genes with moderate effects on disease risk. In that sense, we have moved from competition to collaboration between research groups and large networks and consortia have been established: the Type 1 Diabetes Genetics Consortium –T1DGC–, the Wellcome Trust Case Control Consortium –WTCCC– and The Genetics Association Information Network –GAIN–.

Finally, new analytical tools have had to be developed in order to tackle the vast amounts of information delivered by high through-put genotyping technologies. Both linkage and association studies become more complex with increasing number of genes being simultaneously included. The risk of false positive results has to be accounted for, interaction analyses are necessary to elucidate combined genetic effects, and new tools for analysis are in continuous development.19-21

Most established risk genes
Major Histocompatibility Complex
The genetic region encoding the human MHC, HLA, also designated as IDDM1, is by far the most solidly estab-

Table 1. Risk of T1D for a given individual according to the family member affected18,170-172

<table>
<thead>
<tr>
<th>Relative risk</th>
<th>Absolute risk (%)</th>
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<tbody>
<tr>
<td>No family history</td>
<td>1</td>
</tr>
<tr>
<td>Mother</td>
<td>2.5-5</td>
</tr>
<tr>
<td>Father</td>
<td>12.5-15</td>
</tr>
<tr>
<td>Sibling</td>
<td>15-22.5</td>
</tr>
<tr>
<td>One sibling + one parent</td>
<td>62.5</td>
</tr>
<tr>
<td>HLA-identical sibling</td>
<td>50</td>
</tr>
<tr>
<td>Monozygotic twin</td>
<td>75-175</td>
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</table>

![Figure 1. Genomic regions linked or associated with type 1 diabetes. With permission from www.t1dbase.org](image-url)
lished risk locus for T1D. It is located on the short arm of chromosome 6 (6p21.3), a dense region of highly polymorphic genes extending for 3.5 Mb. The classical class I HLA loci (telomeric) comprise HLA-A, -B, and -C, and class II (centromeric) comprise HLA-DR (DRA, DRB), -DQ (DQA, DQB) and -DP (DPA, DPB). The class III HLA region (located between class I and class II) includes the complement genes C2, C4, Bf, heat shock proteins genes (HSP70), tumour necrosis factor genes (TNF), 21-hydroxylase (21-OH) and others (figure 2).

HLA accounts for up to 50% of the genetic risk, with major effects attributed to certain DR and DQ genes. However, due to high linkage disequilibrium in the region (DR and DQ alleles tend to be inherited together) it is difficult to determine those responsible for the risk. Indeed, associations of HLA alleles with T1D should be considered haplotype specific rather than allele specific. A recent meta-analysis of 38 studies confirmed that haplotypes comprised of DRB1*0401, *0402 or *0405 and DQB1*0301 (DR4-DQ8) were associated with the highest risk of T1D, followed by DRB1*0301 DQB1*0201 (DR3-DQ2) and DRB1*0404 DQB1*0302. The highest risk of T1D is given by the combination of DR3 and DR4 in one genotype. The “neutral” category of haplotypes includes DRB1*0800 DQB1*0402, DRB1*0901 DQB1*0303 (DR9) and DRB1*0100 DQB1*0501 (DR1) and the most protective includes DRB1*1400 DQB1*0503 and DRB1*1500 DQB1*0602 (DR2).

Results from the T1DGC expand these haplotypes to include DQA1, as well as DRB1 and DQB1 (table 2). Their results also indicate that the risk associated with certain HLA haplotypes can be influenced by the genotypic context, as exemplified by the high risk conferred by the trans-complementing DQ heterodimer encoded by the DQA*0501 allele on DR3 and the DQB1*0302 on some DR4 haplotypes.

In Spain, unlike in other European populations, DR3 seems to confer a higher risk of TID than DR4 according to most, albeit not all of the studies. These findings may be due to the particularly high-risk haplotypes associated with DR3 in Spain.

Although DR and DQ alleles confer most of the genetic risk for T1D, class I alleles and maybe other loci within the MHC region are also important in the development of the disease. Further genotyping of class I alleles in
carriers of high-risk class II HLA haplotypes has given further insight into this matter. Siblings of patients with T1D, identical for their DR3/DR4-DQ8 genotypes, who share both HLA haplotypes (defined by HLA A and B, too) with the proband, have a 55% risk of developing T1D by the age of 12 (vs 5% of those sharing 1 or no haplotype). Furthermore, the remarkably conserved HLA A1-B8-DR3 haplotype seems to reduce the risk conferred by DR3-DQ2. Several other reports support an effect of HLA class I genes on disease susceptibility, in particular the HLA-A*2401, *0101 and *0302 and HLA-B*39 alleles. A highly conserved haplotype (B18 AH 18.2), particularly prevalent in the Spanish population, modulates the risk conferred by the DR3-DQ2 alleles. In fact, markers included in the HLA class III region (BAT-2*2 and TNFa2bI) have been found to predict risk in carriers of DR3-DQ2, though it is uncertain whether this effect persists after accounting for class I genes. In addition, a recent report from the T1DGC MCH fine-mapping project identifies additional risk single-nucleotide polymorphisms (SNPs) in the vicinity of HLA-G, COL11A2 and RING1. Other genes within the MHC region that have been reported to be associated with T1D include MIC-A and ITP3, but the results have not been replicated when LD with other class I and class II genes has been taken into account.

Finally, a new locus telomeric to MHC (UBD/MAS1L) has shown to predict development of type 1 diabetes independently of class I and class II genes. Replication in other populations, as well as functional studies of the candidate genes should provide information about their role in the pathogenesis of the disease.

**Non-MHC genes**

Although HLA is responsible for up to 50% of the genetic risk of T1D, other genes are involved, too, albeit with a weaker effect (odds ratios in the 1.15-1.3 range). Most of these genes play important roles in immunity and many are not specific to T1D, but also influence risk for other autoimmune diseases (table 3).

**INS**

The insulin gene (INS), located on chromosome 11p15.5 (IDDM2), is expressed in β-cells and in the human thymus, an expression site likely to be involved in immune tolerance. Two recent studies confirmed that insulin is a major and primary target for autoreactive T-cells both in humans with T1D and in NOD mice. The susceptibility locus IDDM2 maps to a minisatellite composed of a variable number of tandem repeat (VNTR) polymorphism situated 0.5 kb upstream of INS, in the promoter region of the gene. In Caucasians, the main classes of VNTR region minisatelites are named VNTR I (26-63 repeating units) and VNTR III (140-210 units) alleles, where the former is associated with the highest risk of T1D. Although the precise function of the VNTR is still uncertain, the feature of the promoter region of INS may influence the binding of the AIRE transcription factor. The latter regulates the expression of self antigens and controls the thymic expression of insulin and the deletion of autoreactive T-cells during negative selection. The development of immune tolerance to insulin may be reduced through reduction of insulin transcription in thymic medullary epithelial cells. Indeed, the level of insulin expression in the human thymus correlates with the VNTR polymorphism, which in turn correlates with diabetes susceptibility. Recent studies of the INS locus showed that the SNPs -2221MspI, -23HphI (A/T) and +1140 (A/C) confer the highest susceptibility to T1D. Because of their strong linkage disequilibrium with the VNTR, these polymorphism can even be used as markers of the latter.

**CTLA4**

The cytotoxic T lymphocyte antigen-4 (CTLA4) plays an important role in immune response regulation and is strongly associated with autoimmune diseases.
CD28 homologue is mapped to the genetic region of the human IDDM12 locus on chromosome 2q31.60 Its exclusive expression on activated CD4+ and CD8+ T-cells carrying CD2861 in mice seems to be controlled by the Foxp3 gene.62-64 CTLA4 appears to attenuate immune responses by several mechanisms. As a negative regulator of T-cell activation, CTLA4 inhibits T-cell co-stimulation through intercellular interaction, competing for CD28 ligands (CD80/CD86) that are expressed on the surface of antigen presenting cells (APCs). Unlike CD28, which contributes to T-cell activation and maintenance of T-cell response (two signal hypothesis),65,66 CTLA4 restricts the initiation and progression of T-cell immunity.67-69 It also inhibits T-cell activation by different intracellular signalling pathways,70,71 mediates apoptosis of activated human T lymphocytes in an antigen-restricted way,72,73 and has an effect on the suppressive activity of regulatory T-cells (Tregs).74-78 The T1D-associated CTLA4 haplotype contains several polymorphisms in tight linkage disequilibrium (LD), any one of which or a combination thereof could determine the functional effect. The SNP +6230G>A (CT60 rs30807243), located in the 3′ flanking region of CTLA4, has been associated with splicing or altered levels of steady state CTLA4 mRNA.59,60 However, recent results82,83 deny the effect of this SNP on modulation of steady-state mRNA of any known CTLA4 isoform but do not rule out its potential role in the development of T1D-mediated autoimmune disease with multiorgan lymphocytic infiltration and tissue destruction.58,80,81

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<table>
<thead>
<tr>
<th>Table 3. Summary of genes associated with the risk of type 1 diabetes</th>
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<tbody>
<tr>
<td><strong>Gene/region</strong> (IDDM)</td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>HLA (IDDM1)</td>
</tr>
<tr>
<td>INS (IDDM2)</td>
</tr>
<tr>
<td>CTLA4 (IDDM12)</td>
</tr>
<tr>
<td>PTPN22</td>
</tr>
<tr>
<td>IL2RA (IDDM10)</td>
</tr>
<tr>
<td>SUMO4 (IDDM5)</td>
</tr>
<tr>
<td>VDR</td>
</tr>
<tr>
<td>IFIH1 (IDDM19) (mda-5 or Helicard)</td>
</tr>
<tr>
<td>KIAA0350 (CLEC16A)</td>
</tr>
<tr>
<td>PTPN2</td>
</tr>
<tr>
<td>UBASH3A (TULA, CLIP4, STS-2)</td>
</tr>
</tbody>
</table>

AID: autoimmune diseases. VNTR: variable number of tandem repeats. ?: not known at the time of the review.
PTPN22
Similar to CTLA4, PTPN22 (protein tyrosine phosphatase non-receptor type 22) is a diabetes susceptibility locus that is shared by several autoimmune diseases. It is located on chromosome 1p13 and encodes a lymphoid protein tyrosine kinase (Lyp). Lyp is expressed in lymphoid T-cells, where it modulates the activation of protein kinases involved in early T-cell receptor (TCR) mediated signalling events, acting as a negative regulator of TCR signalling. The Arg620Trp (1858C/T) SNP in PTPN22 appears to promote immunoregulation in multiple ways, though there is only limited data regarding the mechanism by which variations in this gene contribute to the pathogenesis of T1D and how they affect immune function in humans. The Arg620Trp variant has been reported to be associated with the development of insulin autoantibodies (in contrast to GAD and IA-2 antibody levels, which were not significantly influenced by this polymorphism) and an accelerated progression of the autoimmune process in T1D. According to two studies, the Arg620Trp variant causes a gain-of-inhibitory function that leads to enhanced suppression of TCR signal transduction, a profound defect in T-cell responsiveness to antigen stimulation and decreased interleukin 2 (IL-2) secretion in T-cells. IL-2 is mainly produced by activated T-cells, promoting their proliferation and expansion. Homozygosity for the Arg620Trp variant is associated with reduced CD4+T-cell and B-cell activation and a shift in the memory T- and B-cell populations to an increase in circulating memory T-cells and fewer and less effective memory B-cells. Lower T-cell signalling may allow the escape of self-reactive T-cells from thymic deletion and lead to impaired self tolerance and reduced Treg development and cause autoimmunity. There have been attempts to assess whether the SNPArg620Trp is the sole T1D susceptibility variant in PTPN22 and, although it seems to be the major risk variant for T1D in this chromosomal region in European populations, the possibility still remains that a yet unidentified variant could have a role in susceptibility to the disease.

IL-2RA
A polymorphism in Interleukin-2 receptor (IL-2RA), located on chromosome 10p15-p14, has recently shown an association with risk of T1D. IL-2RA encodes the α chain of the high-affinity heterodimeric IL-2 cytokine receptor (CD25), highly expressed by activated Tregs. Genetic evidence also suggests a crucial role for IL-2 in T1D. Located on chromosome 4q27, IL-2 plays a critical role in programming T-cells for activation-induced cell death and interferes with immune regulation. IL-2 or IL-2RA knock-out mice completely lack Tregs and suffer from a lymphoproliferative syndrome and spontaneous autoimmune disease. The association of IL-2 and IL-2RA to T1D is probably due to reduced proliferation of a variety of lymphocytes, including Tregs, whose depletion and altered function directly contribute to T1D pathogenesis. Still, the precise role by which IL-2RA/IL-2 are involved in T1D susceptibility needs to be evaluated.

VDR
Epidemiological studies show differences in T1D incidence which are associated with geographical latitude and sun exposure and vitamin D intake before and during infancy seems to protect against the disease. In vitro, vitamin D suppresses the antigen-presenting capacity of T-cells and dendritic cells. In fact, down-regulation of MHC class II molecules and adhesion molecules and inhibition of IL-12 is seen after treatment with vitamin D or its analogues. The relationship between Vitamin D and autoimmune diseases has been thoroughly reviewed elsewhere.

Vitamin D actions are mediated by the highly polymorphic vitamin D receptor which is encoded by VDR, located on 12q12-14. The FokI polymorphism in exon 2 leads to a shorter and more active form of VDR protein, and displays a different distribution in subjects with T1D and controls. However, genetic studies are somewhat contradictory, with some showing an association between VDR variations and T1D, whereas others do not. Population heterogeneity and differences in exposure to ultraviolet radiation may account for these differences. A recent meta-analysis of 16 studies in 19 regions showed an interaction with ambient winter ultraviolet radiation and association between certain VDR alleles and T1D. This study supports the role of vitamin D in the development of T1D, though further studies combining genetic data and information on exposure to ultraviolet radiation and vitamin D status should further clarify this matter.

SUM04
The association of the small ubiquitin-like modifier (SUMO) gene, SUM04, located in the IDDM5 locus on chromosome 6q25, has been confirmed in Asian populations despite controversial observations in Caucasians. A single amino acid substitution (163A>G, MetVal) at an
evolutionarily conserved residue of SUMO4 enhances NFκB transcriptional activity and IL-12B expression and is associated with increased risk for T1D.136,137

Other genes and regions
Interferon induced with helicase C domain 1 gene (IFIH1), involved in anti-viral response, is the most plausible risk gene for T1D in a linkage disequilibrium block on chromosome 2q4 (IDDM19),138-140 though its association with other autoimmune diseases, like Graves' disease141,142 and multiple sclerosis,143 is controversial.

A number of other associations with type 1 diabetes have been identified and replicated for SNPs in two regions on chromosome 12, 12q24 near C12orf30 and 12q13 near erythroblastic leukemia viral oncogene homolog 3 (ERBB3), a region on 16p11 near protein tyrosine phosphatase, nonreceptor type 2 (PTPN2), a region on 2q12-22 encoding interleukin 1 receptor 1 and a region on 21q22.3 in the ubiquitin associated and SH3 domain containing, A (UBA-SH3A, STS/TULA family) locus.22,112,144-148 Many other chromosomal regions are likely to be discovered during ongoing analysis of the data obtained from the WTCCC, T1DGC and GAIN consortia. Detailed and updated information about T1D associated loci can be found in T1DBase (www.t1dbase.org ).

Additional candidate genes
A different approach to identifying new genes involved in T1D consists of trying to replicate results found in closely related diseases, such as T2D, autoimmune diabetes presenting as part of a syndrome and other autoimmune diseases.

Several attempts have been made at associating T2D genes to T1D, but most have been negative.149,150 Patients with autoimmune diabetes diagnosed after the age of 35 and with a clinical presentation more close to T2D (LADA) are an exception. These patients have features of both type 1 and type 2 diabetes, clinically as well as genetically.151-153

Autoimmune diabetes is also a minor component of severe monogenic syndromes such as APS-I or IPEX. APS1 (autoimmune polyglandular syndrome type 1) or autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED)154-156 is a recessive disease caused by mutations of the AIRE gene,157 located on 21q22.158 which encodes a transcription factor with an important role in promoting tolerance and preventing autoimmunity.159-161 Children with the IPEX syndrome (immune dysregulation, polyendocrinopathy, enteropathy, X-linked), also termed the XPID syndrome (X-linked Polyendocrinopathy, Immune Dysfunction and Diarrhoea), present with severe multiorgan autoimmunity (including diabetes) and lymphoproliferation which may lead to death in the first 2 years of life,162 caused by regulatory T-cell failure due to mutations in FOXP3, located on Xq11.23-Xq13.3.163-165

Perspectives
At present, the clinically useful application of genetics in type 1 diabetes is limited to identifying monogenic forms of diabetes where an atypical clinical presentation of T1D (eg: in the first 6 months of life, with a strong family history of diabetes or as part of a distinct syndrome) suggests a monogenic etiology of the disease and where treatments other than insulin may be considered. In addition, identification of individuals at a high risk of developing T1D may be of clinical interest in the future, if interventions that are presently being tested in clinical trials166-168 prove to be safe and effective.

HLA genotyping is a complex process, due to the highly polymorphic nature of the genetic region. Taking advantage of the high linkage disequilibrium in the HLA region, high-risk HLA can be detected by simply analysing a few SNPs169 and thus, high-risk individuals, candidates for intervention, could be easily identified. The already established markers of disease plus the discovery of additional ones may allow further definition in risk assessment. Nevertheless, for the time being, genetic counselling to families of patients with T1D cannot be accompanied by preventive measures.

Conclusions
T1D is a complex autoimmune disease with a proved genetic background. Most (around 50%) of its genetic risk is accounted for by class I and class II HLA haplotypes, although other genes with a smaller effect are also involved. Most of the known risk genes for T1D play a role in immunity, mostly through T-cell regulation (CT-
Practical considerations

- Genetics can improve risk prediction in type 1 diabetes.
- HLA haplotypes can explain the main part of genetic risk in type 1 diabetes.
- Due to the lack of efficient intervention protocols/measures, routine genotyping of type 1 diabetes patients and relatives is not recommended at present.

LA4, PTPN22, IL-2RA and cytokine production or modulation (VDR, SUMO4). The insulin gene (INS) represents and exception to this, and is probably the only gene specifically associated with T1D and not with other autoimmune diseases.

Ongoing genome-wide association studies are providing evidence of multiple known and previously unknown risk genes. New analytical tools are continuously being developed to handle the vast amounts of data produced, as well as to account for multiple comparisons and assess combined effects such as gene-gene and gene-environment interactions.

Acknowledgements:
JCW is being supported by a research grant from the EFSD/ JDRF/ Novo Nordisk Programme 2008.

Potential conflicts of interest
The authors are not aware of any conflicts of interest related to the subject of the review.

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