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Update on Genetic Determinants of Type1 Diabetes

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Abstract

Type 1 diabetes (T1D) results from a progressive destruction of insulin-secreting β cells with consecutive life-long dependence to exogenous insulin. Avoidance of end-stage β-cell mass destruction through primary and secondary prevention strategies requires understanding of initial molecular events leading to insulinopenia. Although autoimmune dysregulation is predominant in T1D, environmental and genetic predisposing factors have been identified and partly account for the heterogeneity of the disease. The use of patient databases and the development of new technologies for genetic screening will help to identify at-risk individuals in the general population or in families with affected siblings. Here we discuss the latest developments in the identification of genetic determinants of T1D and their use for evaluation of disease risk.

Keywords: Type 1 Diabetes; Hyperglycemia; Insulinopenia; Autoimmune disease; Proteiform disease

Introduction

Type 1 diabetes (T1D) is characterized by a progressive destruction of insulin-secreting β cells that results in the development of chronic hyperglycemia and ketone body formation. Because of insulinopenia, restoration of normal glucose homeostasis requires exogenous insulin administration, a feature that defines T1D as insulin-dependent diabetes mellitus (IDDM). Although we still largely ignore the nature of triggering events leading to β-cell demise, T1D is considered an autoimmune disease since the discovery of islet infiltration by lymphocytes (the so-called insulitis) [1] and of islet cell auto-antibodies [2] that are present in 90% of patients [3]. However, recent studies reveal T1D as a proteiform disease [4] and question autoimmune dysregulation as a unique determinant of the disease [5]. Indeed, histological findings shows that insulitis is not consistent in T1D patients [6] and that β-cell mass might endure much longer that initially thought [7,8]. Furthermore, a major argument challenging T1D as an autoimmune disease is that immunomodulation strategies are so far globally inefficient in impeding β-cell destruction [9]. Environmental factors (e.g. diet, viral infection, microbiota) may also be implicated in T1D etiopathogenesis and are increasingly studied because of the disparity of T1D incidence [10] and its secular evolution [11]. However, univocal identification of a self-sufficient causative agent of β-cell death is still pending.

On the other hand, genetic factors have long been implicated in T1D since monozygotic twins have a concordance of the disease of 30-60% [12]. Among genetic factors, HLA class II genes are responsible for most of the risk (30-50%) while other factors exist and confer a higher risk (up to 4 fold) in children with a first-degree relative affected by the disease [13]. Understanding the molecular bases of T1D will provide important tools for the development of primary or secondary prevention strategies. In this review, we discuss the latest developments in the identification of genetic factors influencing the risk of developing islet autoimmunity and T1D.

HLA system

The importance of the HLA locus (chromosome 6p21) on the risk of T1D is known for long [14] and its role has been extended to predisposing and protective haplotypes. The susceptibility haplotypes carried by 90% of European children with T1D are DR3 (*DRB1*0301*) – DQ2 (*DQA1*0501-DQB1*0201*) and DR4 (*DRB1*0401*) – DQ8 (*DQA1*0301-DQB1*0302*) [15] while 30-40% of patients with T1D carry both haplotypes as compared to 2.4% in the general population. The major protective haplotype, present in 15% of Europeans but in <1% of patients with T1D, is DR15 or DR2b (*DRB1*1501*) – DQ0602 or DQ6.2 (*DQA1*0102-DQB1*0602*) [16]. This "DR15-DQ6" haplotype is dominant since it confers protection toward T1D whether or not it is combined with susceptibility haplotypes [17] or even with circulating islet cell antibodies [18]. Associations between HLA class I genes (e.g. HLA-A*2, HLA-A*24 and HLA-B*39) and risk of T1D are also established, yet their influence might depend on the patient's non-HLA class I genetic background. For example, HLA assays in Finnish families with an affected child (n=1764) supported an additive risk conferred by HLA-B*39 [19], whereas Mbunwe et al. [20] described HLA-A*24 and HLA-B*18 – but not HLA-B*39 – as susceptibility alleles in a Belgian cohort. In both studies, T1D risk conferred by HLA class I was observed only in association with specific and distinct HLA class II molecules.

Even though a strong association exists between HLA class II molecules and T1D risk, the identity of high-risk genotypes are changing with time. Studies comparing cohorts of childhood-onset T1D collected in the 60s and the 2000s showed a 30 to 65% decrease of HLA-DR3/4–DQB1*0302 genotype in the latest cohorts [21,22]. In parallel, the rising incidence of T1D is correlated to a higher incidence of lower-risk DR3 and DR4 genotypes [21]. Additional studies on large cohorts will be needed to confirm these trends on a broader array of

genotypes and determine whether this shift in genetic susceptibility of T1D may be accounted to environmental changes.

The role of HLA molecules in developing anti-islet autoimmunity may be estimated via genetic analysis but also by studying polymorphisms in HLA epitopes, which represent variations in the amino acids that line the groove of class I and class II molecules. These amino acids in specific positions can be shared by different HLA alleles. To determine the susceptibility to T1D conferred by shared epitopes, Roark et al., in the context of the HLA Epitope Analysis Program, analyzed the frequency of amino acids in HLA molecules at positions 2–192 (class I) and positions 8–93 (class II) in 830 patients with T1D and 968 control subjects [23] (mostly of North-American origin). Both single and associations of up to four noncontiguous amino acids were tested. In this study, the hierarchy of susceptibility was dominated by A57 epitope of DQB1 (included in five *02 and *03 alleles) responsible for the highest risk (OR=12.1), by four DQA1 epitopes (S26, Q47, R56, and V76, OR=7.15), and by two DRB1 epitopes (H13 in 13 *04 alleles, OR=5.6; K71 in 7 *03, *04 and *13 alleles, OR=3.6). The predominance of DQB1 A57 epitope confirms the β57 hypothesis which associates mutations of aspartic acid at position 57 (Asp 57) of HLA-DQ class II β-chain with susceptibility to T1D, and whose mechanism implicates HLA-DQ instability [24]. The other susceptibility epitopes explain why Asp 57 is retained in DR3 and DR4 risk alleles. The strongest resistance-associated epitopes were DQB1 D57, DQA1 Y80, DRB1 R13, and DRB1 A71 (OR=0.1-0.2), and except DQA1 Y80, all located at the same positions than strong susceptibility epitopes. Consistently with single epitope analyses, homozygosity for DQB1 A57 and D57 respectively gave highest susceptibility and resistance risks when compared to other patterns of HLA epitope inheritance. Also, HLA class I molecules contained low risk susceptibility epitopes (HLA-B C67, OR=1.93; and HLA-C YY9, 116, OR=1.77) but no resistance-associated epitopes. Interestingly, when authors analyzed combinations of two or more amino acids, they did not find significant association neither for susceptibility or resistance.

Moreover, multiple HLA alleles may work together to increase the risk of T1D. Recently, researchers from the Type 1 Diabetes Genetics Consortium evaluated the role of secondary HLA-DRB loci in T1D risk through association studies in a case/control cohort and in T1D families [25]. Using next generation sequencing, they observed that in DRB1*03:01/*03:01 homozygotes, the presence of one or two DRB3*02:02 alleles conferred a higher risk than haplotypes carrying two DRB3*01:01 alleles (OR=25.5 vs 3.4, respectively). Furthermore, the DRB3*02:02 allele was more frequent in homozygous DRB1*03:01/*03:01 patients with T1D (42.9%) than in the nontransmitted DRB1*03:01 chromosomes from heterozygous parents. This study suggests that specific combinations of alleles at a variety of HLA loci explain the risk of T1D conferred by a given HLA haplotype.

The risk conferred by HLA molecules may also depend on associations with other structural changes. In order to test whether modifications of autoantigens may favor autoimmune responses in HLA-DQ8 background, van Lummel et al. [26] provoked posttranslational changes of islet autoantigens using tissue transglutaminase. They observed not only that deamidation of the antigens improved their binding capacity to HLA-DQ molecules, but also that modification of proinsulin activated CD4 T-cell responses with distinct IFN-γ and IL-10 secretion profiles in T1D subjects as compared to healthy controls. Not only antigen processing but also presentation on HLA molecules might influence the role of a given HLA molecule to elicit autoimmune responses. As such, HLA class II molecules bind peptides originating from foreign and self-proteins processed by antigen presenting cells. The non-polymorphic MHCII-like molecule HLA-DM (DM) catalyzes peptide exchange and editing on classical HLA class II molecules, thus controls the peptide repertoire bound to these molecules. A recent study on non-obese diabetic (NOD) mice, a validated murine model of autoimmune diabetes, showed the important role of H-2M molecule, the mouse HLA-DM counterpart, in T1D pathogenesis [27]. In their study, DM alpha-deficient mice had defective class II peptide occupancy and surface expression, and increased proportions of CD4+Foxp3+ regulatory T cells. Most importantly, DM alpha-deficient animals had lower frequency of CD4+ T cells and of insulitis on pathological analyses, and were completely protected against T1D. These data support the hypothesis that inefficient DM editing may play a critical role in T1D-associated autoreactive CD4+ T cell development. Together, these studies suggest that specific patterns of triggering events eventually leading to loss of self-tolerance contribute to the susceptibility provided by HLA molecules (Figure 1).

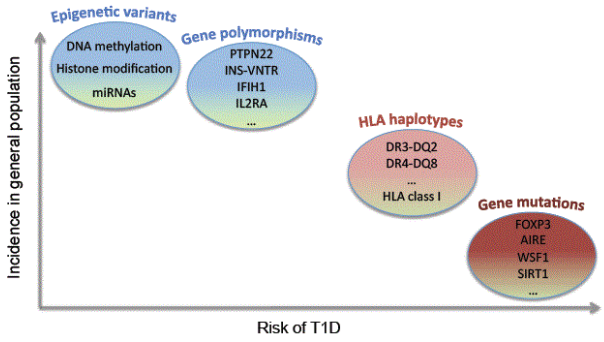

Figure 1: Stratification of T1D risk depending on modifications of the genome code.

Weight gain is another factor that modulates the risk conferred by HLA. In an Australian cohort of 548 infants with a first-degree relative with T1D, Couper et al. [28] observed that 46 children having one or more anti-islet antibodies had higher BMI z-score than at-risk patients not having developed autoimmunity (even when controlling for HLA genotype), and that weight gain was the most predictive of the risk during the first 2 years of life. Recenly, Fourlanos et al. [29] extended these results to adults with latent autoimmune diabetes of adults (LADA), a slowly progressive form of T1D. They found, while comparing high-risk (HLA-DR3,4; DQ2,8) with lower-risk (HLA-DR4,4 or X, DR3,3 or X) patients (n=62), that BMI z-score was higher in lower-risk than in high-risk LADA patients (mean 27.6 vs 24.7 kg/m2), suggesting that inflammation associated with higher body weight or obesity might decrease the requirement for high-risk HLA genes for activation of autoimmune beta cell destruction. Noteworthy, weight gain per se does not seem to confer a risk of islet autoimmunity, as suggested by a study by Cambuli et al. [30] showing no difference in prevalence of anti-islet antibodies in obese or overweight vs normal weight children. Although HLA genotypes were not evaluated in this study, evidence was provided that weight gain may influence autoimmunity on at-risk background only.

The risk of developing T1D conferred by HLA haplotypes and alleles may not be uniform throughout ethnic groups. Indeed, a recent genetic study in African American patients with T1D (n=772) showed that only 12% of these patients had DR3/DR4 genotypes [31]. Furthermore, the African-specific "DR3" haplotype DRB1*03:02-DQA1*04:01-DQB1*04:02 was protective for T1D by opposition to the common high-risk DR3 DRB1*03:01-DQA1*05:01-DQB1*02:01 haplotype. This study showed unique high-risk genotypes, as the heterozygous DR4/DR9 genotype (OR=30.88) that contains the African-derived "DR9" haplotype DRB1*09:01-DQA1*03:01-DQB1*02:01g, and demonstrated that African Americans have a much larger catalog of HLA haplotypes than European (respectively 144 vs 44 haplotypes appearing at least twice).

Gene mutations

Only few gene mutations were directly associated with the development of T1D. FOXP3 (Xp11.23) mutations are known to induce immunodysregulation–polyendocrinopathy–enteropathy–X-linked (IPEX) syndrome that associates recurrent infections, chronic diarrhea, failure to thrive, and endocrinopathies including T1D, which also occur as part of an autoimmune polyglandular syndrome (PGS1) caused by AIRE gene (21q22.3) anomalies, or in Wolfram syndrome (WFS1, CISD2).

Recently, Biason-Lauber et al. [32] identified a family of four probands with T1D that carried a point mutation in SIRT1 gene (exon 1, c.[320T > C]) coding for a histone deacetylase that regulates insulin secretion and cytokine production. Loss-of-function studies conducted via transduction of SIRT1-L107P in MIN6 cells led to increased expression of cytokine-induced NO synthase and production of nitric oxide derivatives, and to increased production of TNF-α and chemokine KC. Furthermore, SIRT1 knockout mice were more sensitive to streptozotocin-induced hyperglycemia than wild-type counterparts. Although the precise mechanisms linking SIRT1 mutation to the autoimmune process are still unknown, this study confirmed the potential to identify single gene implications into the pathogenesis of T1D.

Gene polymorphisms

The development of genome wide association studies (GWAS) introduced the concept of polymorphisms in disease prediction models and broadened the array of loci providing susceptibility to T1D. Exhaustive description of SNP variants associated with T1D was provided elsewhere [33,34].

Among polymorphic genes, variants of PTPN22 are those associated with the highest risk of T1D (about 2 fold). PTPN22 (protein tyrosine phosphatase nonreceptor type 22), on 1p13, codes for a lymphoid tyrosine phosphatase (LYP) regulating TCR signaling and T-cell differentiation [35]. Knockdown studies recently showed the role of PTPN22 in the induction of FOXP3+ regulatory T cell [36] and variants of the protein are known causative of systemic auto-immunity [37]. The variant C1858T, characterized by a nonsynonymous substitution of an arginine to a tryptophan at codon 620 (rs2476601) affecting its catalytic activity [38], is responsible for an increased risk of T1D (OR up to 3.6) across all populations [35,39], yet other variants may confer a risk in specific populations [40]. Modulation of PTPN22 expression in the NOD mouse model may result in attenuation of the T1D phenotype, as shown recently on transgenic NOD mice overexpressing PEST domain-enriched tyrosine phosphatase in T cells [41]. This resulted in reduced TCR-mediated effector cell responses, increased proportions of CD4+FOXP3+ cells and splenocytes with lower diabetogenic ability. Consistent with these results, Zheng et al recently described that the PTPN22 susceptibility allele C1858T (LYP R620W in human, PEP 619W in mouse) might be a gain-of-function variant [42]. Using an RNA interference system against PTPN22 under the control of a tetracycline-inducible promoter inserted in NOD zygotes, the authors noticed higher peripheral but not thymicTreg cells and decreased survival of B cells, both features opposite to those observed in the human phenotype. Most importantly, the PTPN22 knockdown protected the transgenic NOD mice against development of autoimmune diabetes. Elucidation of the impact of PTPN22 R620W variant on its function is critical for development of drug screening programs [43].

Accordingly to the central role of insulin protein as auto antigen in T1D pathogenesis, variants in INS gene (11p15.5) may confer a 2-fold risk of T1D [44]. A polymorphism corresponding to a variable number of tandem repeats (VNTR) exists upstream of the INS promoter. In this IDDM2 locus, short numbers of repeats (VNTR class I) increase the risk of T1D whereas longer repeats (VNTR class III) are protective. Shorter number of repeats affects transcriptional activation of the INS promoter by AIRE and thusthymic expression of insulin and its precursor proteins, which in turn leads to a decrease in thymic deletion of insulin-specific auto-reactive T cells [45]. Polymorphisms at the IDDM2 locus act independently of the HLA risk alleles [46].

Enter viruses have for long been regarded as a putative primummovens to islet autoimmunity [47]. Infection of pancreatic β cells by enteroviruses stimulates viral replication and the resulting production of double-stranded (ds) RNA activates innate immune responses (production of type I interferons and NFκB) that are mediated by the interferon-induced with helicase C domain 1 (IFIH1) protein. The extent of activation of these innate mechanisms of dsRNA recognition correlates with various levels of upregulation of HLA class I expression by β cells, which may then become targets of autoimmune attack and destruction by CD8+ T cells. Levels of IFIH1 activity thus influences auto-antigen presentation by β cells, and the major nonsynonymous SNP (rs1990760, A946T) in IFIH1 conferring a risk of T1D has first been described in 2006 [48]. Whether polymorphisms in IFIH1 may participate in the susceptibility toward other autoimmune diseases is still under debate [49-51]. The susceptibility conferred by IFIH1 allele variant rs1990760 is itself low (OR=0.86), as is the protection associated with rs35744605, rs35337543, rs35732034 and rs35667974/Ile923Val variants (OR=0.51-0.74) [52,53]. However, activation of interferon pathways has been recognized as a trigger of islet autoimmunity in at-risk populations. In a recent study on 64 patients with T1D, 109 genetically at-risk children and 87 healthy controls, Ferreira et al. [54] analyzed the expression of 225 IFN-β inducible genes initially identified through microarray after in vitro studies. They observed that this set of genes – to which IFIH1 belong – was transiently activated after an upper respiratory tract infection and that this activation preceded the appearance of islet autoimmunity. Basal levels of expression recovered once β-cell destruction occurred.

The interleukin (IL)-2 pathway is also involved in T1D risk and this is linked to the role of IL2 in the survival and expansion of Tregs (CD4+CD25+FOXP3+ T cells) [55]. Tregs require binding of IL2 to its receptor – and its high affinity α subunit or CD25 encoded by IL2RA – on their membrane to exert their suppressive function via activation of FOXP3. Any deficiency in this mechanism may result in persistence of

circulating autoreactive T cells and foster development of autoimmune diseases [56]. Accordingly, allele variants in IL2RA have been identified as risk factors for T1D [57-59] while protective haplotypes have also been described [60]. The IL2RA risk allele rs12722495 was associated with lower IL-2 signaling and Treg function [61]. Encouraging preclinical data in NOD mice suggested that forced IL-2 expression in β cells could suppress the development of T1D by recruiting FOXP3+ Tregs [62]. Ongoing clinical trial with low-dose IL-2 regimen in overt T1D patients [63] will provide better insight in the feasibility to exploit IL-2/IL-2RA pathway in alleviating autoimmune pressure [64].

Another molecule implicated in the regulation of immune responses and providing a risk for developing T1D is cytotoxic T lymphocyte-associated protein 4 (CTLA-4) (2q33) encoded by CTLA4 on chromosome 2q33 in the IDDM12 locus. CTLA-4 is a glycoprotein receptor expressed on activated T cells that plays a role as a suppressor of T-cell activation which happens when T cells encounter a (self)-antigen and bind B7 (CD80 and CD86) on antigen-presenting cells via their CD28 receptor. CTLA4-/- mice develop severe lymphoproliferative disorders [65]. Although the exact mechanisms linking CTLA-4 to autoimmune dysregulation are not clarified yet, CTLA4 main polymorphisms – G49A (rs231775) and C60T (rs3087243) – associate with T1D with OR of 1.42 and 1.23, respectively, according to a recent meta-analysis on 58 studies including 30,723 cases of T1D and 25,254 controls [66].

Epigenetic factors

Epigenetics influences the way inherited gene expression varies among individuals without modification of DNA sequence. Concretely, it mainly corresponds to influences on the genetic code exerted by DNA methylation, histone modification and non-coding RNAs. In the context of T1D, differences between patients and controls in CpG methylation patterns have been described for INS, HLA-DQB1, and GAD2 genes [34]. More recently, a study on a French cohort of 252 patients with T1D and 286 controls identified different SNPs at two CpG sites of the proximal promoter region of IL2RA associating with T1D and that could at least partly explain the risk conferred by IL2RA alleles. miRNAs are other non DNA-regulators of gene expression through their binding to 3'UTRs of mRNAs and the resulting repression of specific targets. Several miRNAs (e.g. miR-25 [67] or miR-146 [68]) are believed markers of β-cell destruction and were identified either in sera or PBMCs of patients with T1D. The search for miRNAs is currently motivated by the potential to precociously identify patients with ongoing autoimmunity [69]. Because of the potentially high number of circulating or lymphocyte-specific miRNAs, the use of large databases, high-power array analyses and computational biology will be required to characterize post-transcriptional events involved in the triggering events of T1D [70].

Calculate the risk

Since the etiopathogenesis of T1D remains largely hypothetical, it is currently arduous to anticipate the onset of insulinopenia in patients with T1D. Identification of at-risk patients is required for the implementation of secondary prevention strategies. The risk of T1D may be evaluated by measurement of islet auto-antibodies (anti-IA2, anti-GAD65, anti-insulin) in the genetically at-risk [71,72] or in the general population [73]. Whereas the presence of a single anti-islet autoantibody is of poor predictive value [74], the more autoantibodies are detected in an individual's serum, the higher is the risk of developing T1D. In the Ziegler et al study combining prospective birth cohorts from various studies [72], T1D risk increased from about 10% at 15 years of age in patients having one single autoantibody to 60% when 2 autoantibodies were detected. Another study from the same group [71] observed a strong correlation between high titers of anti-insulin and anti-IA2 antibodies and T1D risk. However, in our experience titers of anti-GAD65 and anti-IA2 at diagnosis of T1D did not correlate to the extent and speed of β-cell mass decline that occurred during partial remission [Pecheur et al., unpublished]. Nevertheless, it is globally accepted that in individuals not having the protective HLA DR2-DQA1*0102-DQB1*0602 haplotype, presence of ≥2 anti-islet autoantibodies confers a risk of T1D that varies from 25% to 50% in the next 5 years [75,76].

The risk of T1D may also be evaluated using HLA typing, as already suggested in the early 1970s [77]. In a longitudinal study on a French cohort of first-degree siblings of patients with T1D, a hierarchy of risk was recognized by HLA-DR3 and DR4 determination [78]. After 8 years of follow-up, the risk of T1D was 16% for DR3/DR4 heterozygotes, 10% for patients with HLA alleles matching the affected parents and 3-4% for those carrying either DR3 or DR4 allele. This is in line with another study on a mostly Caucasian cohort showing a 20-fold increased risk associated with DR3/DR4 heterozygosity [79]. The potential for fine-tuning of risk determination has been evaluated using co-analysis of HLA and non-HLA susceptibility loci. By using the Bayesian Network probabilistic approach, a recent study associating a case-control cohort and a family data set (total of about 5000 French individuals) showed that INS-VNTR and PTPN22 susceptibility polymorphisms only marginally influenced the risk conferred by HLA-DRB1 [80]. However, in a prospective study on 1,650 children from the BABYDIAB study (children born to T1D-affected parents from Germany), Achenbach et al. [81] showed that the non-HLA loci IL2, CD25, INS VNTR, IL18RAP, IL10, IFIH1, and PTPN22 were useful for discrimination between rapid and slow progressors, whereas HLA-DR/DQ genotypes and autoantibodies were equally balanced in the subgroup of patients that developed autoimmunity no matter how fast they evolved toward overt diabetes. The implication of non-HLA determinants of T1D risk was further supported by a longitudinal study that followed up to adulthood 2,134 siblings of probands with T1D (95% Caucasian) [82]. In these families, DR3-DQ2/DR4-DQ8 was responsible for a cumulative risk of T1D of 17% at age 15, but the age at diagnosis of the proband with diabetes was much more determinant as younger ages (<10 years) were associated with a cumulative risk of 61% at age 15 (vs 4.7% for older age probands).

Finally, combination of several screening methods might be the most refined approach to identify patients susceptible to develop overt T1D. This is suggested by the study of Ferrannini and colleagues who evaluated insulin secretion and glucose sensitivity in relatives of patients with T1D [83]. They observed that although patterns of insulin secretion and sensitivity were similar between progressors and non-progressors, a decline of β-cell glucose sensitivity – determined by integration of insulin and glucose concentration curves during a 2h OGTT – predicted the apparition of the disease. Additional studies are required to confirm the power and the usefulness of combining genetic and in vivo studies for risk determination of T1D in the setting of clinical routine.

Conclusion

T1D is a complex disease determined by variable evolution of the function of β cells that are per se highly sensitive to stress induced by the immune system and/or environmental agents. Analysis of determinants of T1D risk in the genome and epigenome will help elucidate the primary events leading to immune dysregulation and β-cell death, and perhaps reveal targets for prevention strategies. Genetic association studies will also improve our potential to identify at-risk individuals that might benefit from these interventions.

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