The role of interferon induced with helicase C domain 1 (IFIH1) in the development of type 1 *diabetes mellitus*

O papel do interferon induzido com o domínio C da helicase 1 (IFIH1) no desenvolvimento do diabetes melito tipo 1

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ABSTRACT

Type 1 *diabetes mellitus* (T1DM) is a chronic, progressive, autoimmune disease characterized by metabolic decompensation frequently leading to dehydration and ketoacidosis. Viral pathogens seem to play a major role in triggering the autoimmune destruction that leads to the development of T1DM. Among several viral strains investigated so far, enteroviruses have been consistently associated with T1DM in humans. One of the mediators of viral damage is the double-stranded RNA (dsRNA) generated during replication and transcription of viral RNA and DNA. The *IFIH1* gene encodes a cytoplasmic receptor of the pattern-recognition receptors (PRRs) family that recognizes dsRNA, playing a role in the innate immune response triggered by viral infection. Binding of dsRNA to this PRR triggers the release of proinflammatory cytokines, such as interferons (IFNs), which exhibit potent antiviral activity, protecting uninfected cells and inducing apoptosis of infected cells. The *IFIH1* gene appears to play a major role in the development of some autoimmune diseases, and it is, therefore, a candidate gene for T1DM. Within this context, the objective of the present review was to address the role of *IFIH1* in the development of T1DM. Arq Bras Endocrinol Metab. 2013;57(9):667-76

Keywords

Autoimmunity; type 1 *diabetes mellitus*; viral infection; IFIH1

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RESUMO

O diabetes melito tipo 1 (T1DM) é uma doença autoimune crônica e progressiva caracterizada por descompensações metabólicas frequentemente acompanhadas por desidratação e cetoacidose. Os agentes virais parecem ter um papel importante no desencadeamento da destruição autoimune que leva ao desenvolvimento do T1DM. Entre as cepas virais estudadas até agora, a família dos enterovírus foi consistentemente associada ao surgimento da doença em humanos. Um dos mediadores do dano viral é o RNA fita dupla (RNAfd) gerado durante a replicação e transcrição de RNA e DNA viral. O gene *IFIH1* codifica um receptor citoplasmático pertencente à família dos *pattern-recognition receptors* (PRRs) que reconhece o RNAfd, tendo um papel importante na resposta imune inata desencadeada por infecção viral. A ligação do RNAfd a essa PRR desencadeia a liberação de citocinas pró-inflamatórias como interferons (IFNs), os quais exibem uma potente ação antiviral e têm como objetivo proteger as células não infectadas e induzir apoptose naquelas já contaminadas. O gene *IFIH1* parece ter uma participação importante no desenvolvimento de algumas doenças autoimunes. Por isso, esse gene é um candidato ao desenvolvimento do T1DM. Dentro desse contexto, o objetivo da presente revisão foi abordar o papel do *IFIH1* no desenvolvimento do T1DM. Arq Bras Endocrinol Metab. 2013;57(9):667-76

Descritores

Autoimunidade; diabetes melito tipo 1; infecção viral; IFIH1

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INTRODUCTION

Type 1 *diabetes mellitus* (T1DM), which accounts for 5%-10% of all cases of *diabetes mellitus*, is characterized by severe insulin deficiency secondary to autoimmune destruction of pancreatic beta-cells. Consequently, subjects with T1DM are usually dependent on insulin injections for life (1-3). Markers of autoimmune beta-cell destruction include autoantibodies to insulin, to the islets of Langerhans, to glutamic acid decarboxylase (GAD65), to beta-cell-specific zinc transporter ZnT8, and to tyrosine phosphatases IA-2 and IA-2β. One – but usually more – of these antibodies are present in 85%-90% of patients when fasting hyperglycemia is initially detected.

Inflammation of the islets of Langerhans (insulitis) probably develops within the context of a "dialog" between immune cells and beta-cells. This dialog is mediated partly by cytokines and chemokines, which are released both by immune cells and by the beta-cells themselves, as well as by other immunogenic signals delivered by dying beta-cells. This may lead to induction and amplification of the inflammatory process, but in some cases, may lead to the resolution of insulitis, instead (2). The course of beta-cell inflammation and its potential progression to clinical T1DM depends on a complex interaction between a strong genetic component and a variety of environmental triggers (4,5). Among the various loci associated with T1DM, the human leukocyte antigen (*HLA*) class II locus on chromosome 6p21 is, by far, the leading genetic risk factor for T1DM, accounting for 30%-50% of genetic risk for the condition (6). Other genes are associated with relatively minor effects on T1DM risk compared with HLA, such as the *insulin* gene, the cytotoxic T-lymphocyte associated protein 4 (*CTLA4*) gene, the protein tyrosine phosphatase, nonreceptor type 22 (*PTPN22*) gene, the interleukin 2 receptor – alpha (*IL2RA*) gene, the interferon induced with helicase C domain 1 (*IFIH1*) gene, and other genes recently discovered by means of genome-wide association studies (GWAS) (Figure 1) (6,7). Winkler and cols. (8) evaluated how the combined allele frequency of 12 T1DM susceptibility genes could stratify T1DM risk in children of parents with T1DM, followed up from birth until the development of islet autoantibodies and diabetes. The authors showed that non-HLA gene combinations were highly effective in discriminating T1DM, and were most effective in children with a high-risk HLA genotype.

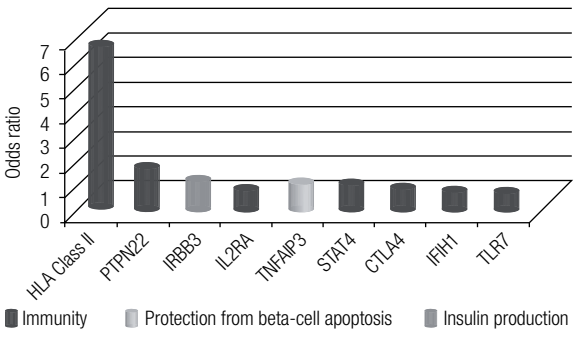

Figure 1. Genes associated with type 1 *diabetes mellitus*. Odds ratios for susceptibility alleles of nine genes associated with type 1 *diabetes mellitus*. Figure adapted from Todd and cols. (7).

The greatest T1DM discrimination was obtained by the sum of risk alleles of *IFIH1*, *CTLA4*, *PTPN22*, *IL-18RAP*, *SH2B3*, *KIAA0350*, *COBL*, and *ERBB3* genes in HLA-risk children.

The environmental triggers and potentiators of autoimmune beta-cell destruction include viral infections, dietary exposure during childhood (*e.g.* to cow milk), vaccination, and toxins (9,10). There is substantial evidence that viral pathogens, such as enteroviruses, rubella virus, mumps virus, rotaviruses, parvoviruses, and cytomegalovirus, play a major role in triggering the autoimmune destruction of pancreatic beta-cells (11,12). Among these viral strains, particular attention is given to enteroviruses, which exhibit specific tropism for the pancreas and have been associated with the development of T1DM in humans (13,14). Epidemiological studies of the seasonality of development of anti-beta-cell antibodies in a group of subjects at increased risk of T1DM showed an increased incidence of autoantibodies during winter, which correlated with a period of increased enteroviral infection rates (10). Furthermore, coxsackieviruses isolated from patients with T1DM were able to induce diabetes in susceptible mice (15). In a recent study, the Coxsackie B4 virus was identified in 50% of samples collected from patients with T1DM and was also able to infect human islets *in vitro*, impairing insulin secretion in response to glucose (16).

Based on the studies presented above, enteroviruses appear to be associated with a fraction of T1DM cases. Nevertheless, if enteroviruses play a major role in T1DM pathogenesis, how could we explain the increase in T1DM incidence in countries where exposure to these microorganisms has been dropping, such as Fin-

land? Are the data showing that T1DM can be caused by viral infections compatible with the hygiene hypothesis (17)? Interestingly, data in NOD mice revealed that coxsackieviruses provoke diabetes only when a preexisting mass of insulitis has accumulated. When administered earlier in the life, inoculation is associated with a strong protection against diabetes (18). Taking into account the studies in NOD mice, Coppieters and cols. (18) suggested that the lack of exposure to enteroviruses in developed countries results in a reduced frequency of subjects with protective immunity caused by early childhood infections. When islet inflammation occurs in these subjects, they would be more susceptible to an enteroviral infection that has the potential to initiate autoreactivity and beta-cell damage.

Microbial recognition by the mammalian immune system relies on components of both innate and adaptive immunity. Innate immunity is the first line of defense against bacteria, fungi, and viruses. Detection of invading microorganisms is carried out by a wide range of cell receptors of the pattern-recognition receptors (PRRs) family, which recognize highly conserved pathogen-associated molecular patterns (PAMPs), such as the double-stranded RNA (dsRNA) generated during viral RNA replication and transcription (19-21). Innate immune system cells, such as macrophages and dendritic cells, kill invading microorganisms by phagocytosis or induction of cytokine production. Furthermore, innate immunity activates the adaptive immune system, consisting of B lymphocytes, which produce specific antibodies against the invading pathogen, and T lymphocytes, which secrete cytokines that will induce elimination of infected cells by exerting cytotoxic effects or by signaling to B lymphocytes (21).

Some studies have shown that certain PRRs, such as IFIH1, play a role in the development of T1DM in animal models (2,22). The *IFIH1* gene, also known as the melanoma differentiation-associated gene-5 (*MDA-5*), encodes a cytoplasmic receptor that recognizes dsRNA and is involved in the innate immune response triggered by viral infection (23). Binding of dsRNA to IFIH1 triggers the release of proinflammatory cytokines, particularly interferons (IFNs), by immune cells, thus inducing apoptosis of virus-infected cells (19,24). Therefore, *IFIH1* is a candidate gene for T1DM susceptibility. Within this context, the objective of the present review was to address the role of *IFIH1* in the development of T1DM.

THE ROLE OF PATTERN RECOGNITION RECEPTORS (PRRS) IN THE RESPONSE TO VIRAL INFECTION

As mentioned above, recognition of pathogens by the innate immune system relies on PRRs, which constitute the first line of defense against microbial infection (25,26). Recent studies have identified three groups of PRRs: toll-like receptors, retinoic acid-inducible gene I (RIG-I)-like helicases (RLHs), and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) (27,28). The RLH class comprises the helicases IFIH1/MDA-5, retinoic acid-inducible gene 1 (*RIG-I*), and Laboratory of Genetics and Physiology-2 (*LGP2*).

During viral infection, dsRNA or single-stranded RNA (ssRNA) are recognized by specific PRRs present on infected cells, which undergo conformational changes and activate signaling cascades that ultimately drive the production of several proinflammatory cytokines, chemokines, and type I IFNs (IFN-I) (19,29). IFN-I is a cytokine produced by most cells during viral infection that promotes the expression of several genes involved with antiviral response in target cells, and acts as a modulator of the adaptive immune system by activating dendritic cells, T lymphocytes, and B lymphocytes (30). This IFN exhibits potent antiviral action, protecting uninfected cells and inducing apoptosis of infected ones, which is partially caused by endoplasmic reticulum stress (27). Interestingly, high levels of IFN-I are found in the pancreas of patients with T1DM, and IFN-α is known to contribute to the development of experimental viral-induced diabetes (2). Furthermore, IFN-I treatment appears to protect against the development of T1DM and reduces the incidence of the disease in NOD mice (31).

Human pancreatic islets infected with the Coxsackie B5 virus or exposed to IFN-α or IFN-γ + IL-1β exhibit increased expression of Toll-like receptor 3 (TLR3) and RLHs (RIG-1 and IFIH1) (32,33). Both intracellular and extracellular dsRNA may bind to TLR3 and trigger the production of proinflammatory cytokines and chemokines, resulting in beta-cell apoptosis by means of the activation of key transcription factors: nuclear factor-kappa B (NF-κB) and IFN-regulatory factor 3 (IRF-3) (34-36). Although TLR3 was the first dsRNA sensor identified as being able to activate NF-κB and IRF-3, its role as a primary antiviral receptor was recently questioned (37). *In vivo* antiviral responses against a wide range of viral pathogens, including the vesicular stomatitis virus, reoviruses, murine cytomegalovirus,

and lymphocytic choriomeningitis virus, were similar in wild-type and *TLR3* knockout mice (38). Indeed, more recent studies show that, whereas NF-κB and IRF-3 activation by extracellular dsRNA is TLR3-dependent, activation by intracellular dsRNA, a product of viral replication in the cytoplasm, also occurs by means of activation of RIG-1 and IFIH1 (34,35). Activation of NF-κB and IRF-3 triggers production of IFN-α and IFN-β, leading to activation of the Jak/STAT-1 pathway and triggering the expression of MHC class I antigens and a variety of chemokines (26,33,35,39). This complex molecular response leads to the attraction of immune cells, which will release more proinflammatory cytokines, such as IFN-γ, IL-1β, and TNF. Local inflammation and activation of antiviral defenses seek to eradicating infection and triggering apoptosis of infected cells. However, in some genetically susceptible individuals, this defense system fails to work properly, inducing excessive, progressive inflammation and prolonged death of beta-cells, instead, thus predisposing to the development of T1DM (2).

THE INTERFERON INDUCED WITH HELICASE C DOMAIN 1 (IFIH1) RECEPTOR

The IFIH1 protein belongs to a family of helicases that also comprises two other members: the RIG-I and LGP2 receptors (26). RNA helicases are highly conserved enzymes that use energy derived from ATP hydrolysis to bind dsRNA, destabilizing and unwinding it (40). IFIH1 and RIG-I contain two CARD (N-terminal caspase activation and recruitment domains) effector domains essential to their signaling activity. Furthermore, all three helicases contain a DExD/H-box-type RNA helicase domain, which is also essential to their function. A C-terminal domain was recently identified as the site of dsRNA binding in all three helicases (26,41,42). The LGP2 helicase does not contain CARD domains and is probably unable to activate downstream signaling pathways. LGP2 also recognizes dsRNA, but appears to act as a negative regulator, interfering with viral RNA recognition by IFIH1 and RIG-I (42). Depending on the type of virus, LGP2 may also act as a positive regulator of its other RLH counterparts (21).

After binding with virus-derived dsRNA, IFIH1 and RIG-I interact, via their CARD domains, with the IPS-1 adaptor molecule (IFN-β promoter stimulator-1, also known as Cardif, MAVS or VISA), which recruits intermediary signaling molecules, such as IKK-α, -β, -ε, and TBK1, ultimately leading to NF-κB and IRF-3 activation (20,26,41,42). Furthermore, TRAF3, an antiviral molecule implicated in the production of IFN-I, also plays a role in these intracellular signaling events (37). As described elsewhere, activation of NF-κB and IRF-3 induces expression of IFN-β and several genes regulated by these transcription factors, which will direct inflammatory and antiviral responses against infection (Figure 2) (26,42).

IFIH1 and RIG-I share a sequence homology of 25% within the CARD domains and 40% within the helicase domain (41). Experimental data suggest that these two helicases use similar intracellular signaling mechanisms to induce an antiviral response. However, despite their structural similarities, IFIH1 and RIG-I appear to be non-redundant, and are involved in the recognition of different types of dsRNA and ssRNA virus. Whereas RIG-I recognizes the hepatitis C virus, several paramyxoviruses (mumps virus, varicella zoster virus, respiratory syncytial virus, parainfluenza virus), vesicular stomatitis virus and influenza A virus, IFIH1 recognizes *Picornaviridae*, such as rhinoviruses, echoviruses, enteroviruses, and encephalomyocarditis virus (23). Furthermore, *Ifih1* knockout mice (Ifih1$^{-/-}$) fail to produce IFN-α in response to the exposure to synthetic dsRNA polyinosinic:polycytidylic acid (PIC), which demonstrates that IFIH1 is the primary cytoplasmic sensor for long PIC (23). Interestingly, these two helicases appear to recognize reoviruses, the West Nile virus, and the dengue virus (26). Experiments involving PIC infection have shown that short dsRNA segments (< 2000 bp) activate RIG-I, whereas long dsRNA segments (> 2000 bp) are best recognized by IFIH1 (43,44). Nevertheless, the mechanisms responsible for distinguishing long dsRNA segments from short ones have yet to be elucidated.

The *IFIH1* gene was identified by subtractive hybridization as a novel gene overexpressed in HO-1 human melanoma cells induced to differentiate by treatment with IFN-β and mezerein, a protein kinase C activator (45). The human *IFIH1* gene is located on chromosome 2 (region 2q24.3), contains 16 exons (Figure 3) and encodes a 1,025-amino acid protein with a molecular mass of 116.7 KDa (42,46). This helicase is expressed at low levels in a wide range of tissues, including pancreatic beta-cells, but is expressed at relatively high concentrations in immune cells (32,46). At the transcriptional level, *IFIH1* expression is induced by IFN-I, retinoic acid, and dsRNA (42).

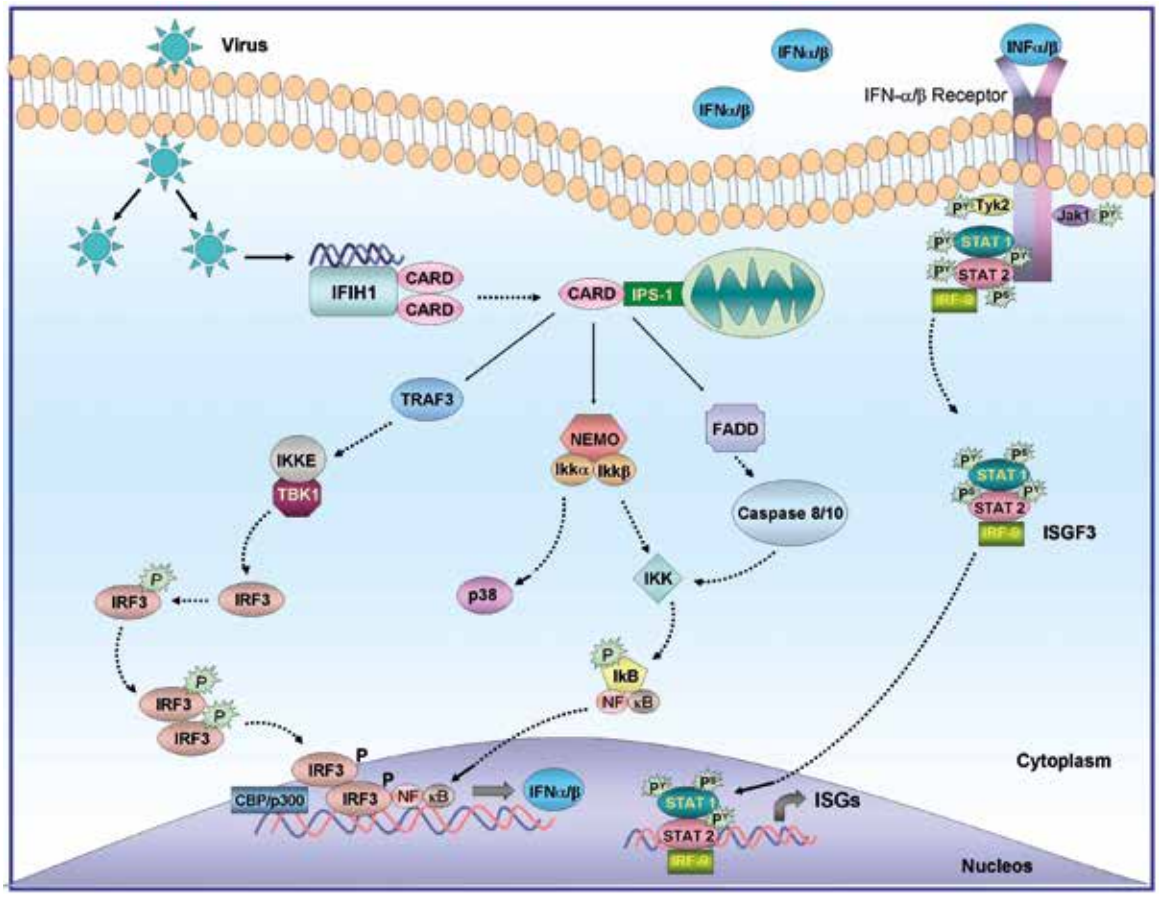

Figure 2. Antiviral signaling by IFIH1. Double-stranded RNA (dsRNA) derived from viral replication is detected by the cytoplasmic RNA helicase IFIH1, activating the adaptor protein IPS-1 via CARD domain interactions. IPS-1 then induces intracellular signaling pathways that result in the activation of the transcriptions factors IRF-3 and NF-κB, leading to the production of IFNα/β by infected cells. IFNα/β is then shown signaling through the IFNα/β receptor and the Jak-STAT pathway to drive interferon-stimulated genes (ISGs) expression and an innate immune response. See text for further details. Adapted from Wilkins and Gale (26).

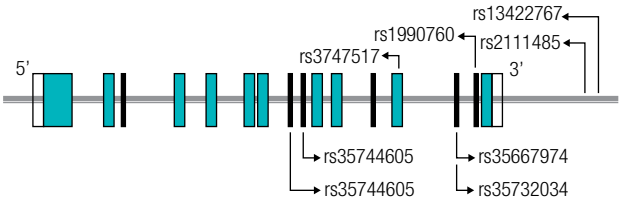

Figure 3. Map of human *IFIH1* locus on chromosome 2 (region 2q24.3). The sixteen exons (boxes) are numbered from left to right according to the transcriptional region. The arrows show the main common polymorphisms associated with type 1 *diabetes mellitus*. Figure adapted from Chistiakov and cols. (42).

Hühn and cols. (47) found that *Ifih1*$^{-/-}$ knockout mice exhibit increased susceptibility to Coxsackie B3 virus infection. Loss of *Ifih1* enabled faster viral replication, leading to hepatomegaly, pancreatic injury, and high mortality rates in these animals. The authors also found that *Ifih1* is not required for induction of IFN-I, but is essential for production of peak IFN-α levels after infection. Furthermore, both Ifih1 and *Tlr3* appear to play a major role in the prevention of diabetes in C57BL/6 mice infected with encephalomyocarditis virus strain D, a beta-cell-tropic virus (48). Deletion of only one allele of the *Ifih1* gene was enough to cause transient hyperglycemia in mice infected with the virus, whereas mice in which both copies of the gene were deleted exhibited severe cardiac disease (48). Colli and cols. (30) assessed the effect of Ifih1 blockade by transfecting primary beta-cells and INS-1E beta-cells (a rat insulinoma cell line) with anti-Ifih1 siRNA and exposing these transfected cells to PIC. As expected, PIC increased *Ifih1* gene expression in these cells, which was inhibited by siRNA. Inhibition of Ifih1 in primary and INS-1E beta-cells did not inhibit PIC-induced apoptosis; however, it did reduce expression of proinflammatory cytokines (IFN-β and IL-15) and chemokines

(CCL2, CCL5 and CXCL10), suggesting that Ifih1 is not essential for PIC-induced beta-cell death, but rather regulates important inflammatory signaling mechanisms in these cells (30).

In mice, increased gene expression of *Ifih1* leads to a state of chronic IFN-I production, characterized by resistance to lethal viral infections (49). Hultcrantz and cols. (32) showed that, in human pancreatic islets, IFN-induced antiviral defenses provide a powerful protective mechanism against replication of coxsackieviruses. Treatment with IFN-α is known to increase gene expression of the chemokine CXCL10 in human islets (32). This chemokine, when produced during infection, leads to T-cell recruitment to the islet site and appears to play a key role in host defense against islet-tropic viruses in individuals susceptible to T1DM (32).

These studies further strengthen the hypothesis that *IFIH1* plays a key role in the regulation of local islet inflammation during viral infection.

IFIH1 gene polymorphisms and their association with T1DM

The association between *IFIH1* and T1DM was first reported in 2006, by Smyth and cols. (50), who conducted a GWAS in European families affected by T1DM within a large Caucasian population cohort from the United Kingdom, for a total of over 10,000 subjects. Several *IFIH1* polymorphisms were associated with T1DM, with the rs1990760 (G/A) polymorphism, which substitutes an alanine to valine in codon 946 of exon 15, being most strongly associated with protection against development of the disease (odds ratio [OR] = 0.86, P = 1.42 x 10^{-10} for the G allele) (50).

Associations between *IFIH1* polymorphisms and T1DM have been replicated in some populations (29,51-55), but not in others (56-58) (Table 1). Jermendy and cols. (52) studied the rs1990760 polymorphism in Hungarians and Finns and conducted a meta-analysis of 5 studies that analyzed this polymorphism in T1DM patients and nondiabetic controls, including their own study. In Hungarians, the A allele of this polymorphism was strongly associated with T1DM (OR = 1.29; P = 0.002). Furthermore, the meta-analysis showed a significant association between the A allele and risk of developing T1DM (OR = 1.18; P = 5.3 x 10^{-15}).

Qu and cols. (53) assessed three single nucleotide polymorphisms (SNPs) located in the *IFIH1* gene or its adjacent intergenic regions (rs1990760, rs3747517, and rs2111485) in 589 French Canadian nuclear family trios. The rs1990760 and rs3747517 polymorphisms showed a trend toward association with T1DM as reported by other studies, but the effect did not reach statistical significance, most likely due to weak statistical power (53). Conversely, the A allele of SNP rs2111485 was associated with protection for T1DM (OR = 0.84, P < 0.05). Yang and cols. (55), in a study of Han Chinese subjects, also failed to find an association between the rs1990760 polymorphism and T1DM, but did find an association between the rs3747517 polymorphism and the condition (P > 0.001).

A GWAS of Caucasian subjects in the United States (Georgia and Denver populations) showed that two SNPs in the coding region of *IFIH1* (rs1990760 and rs35744605) and two SNPs in the adjacent 3' intergenic region (rs2111485 and rs13422767) were associated with increased risk of T1DM (OR = 1.7-1.9), but only in the Georgia population, with the lowest P-value obtained for the rs1990760 polymorphism (P = 8 x 10^{-8}) (51). Interestingly, the G/G genotype of SNP rs1990760 was associated with increased levels of *IFIH1* expression in the peripheral blood mononuclear cells of 374 subjects (187 patients with T1DM and 187 nondiabetic controls) (51). The most common homozygous genotypes for the three other polymorphisms of interest were also associated with increased *IFIH1* expression, which suggests that increased expression of this gene may be associated with greater susceptibility to T1DM development (51). Nejentsev and cols. (29) reported that four rare variants of the *IFIH1* gene (rs35337543, rs35667974, rs35744605 and rs35732034), as well as the rs1990760 polymorphism, were independently associated with protection against T1DM in a British population (OR = 0.51-0.84; P = 1.3 x 10^{-3} to 2.1 x 10^{-16}).

The rs1990760 polymorphism is not located in any functional region of the protein (42), but the G allele is highly conserved among mammals and may have other, yet-unknown functions, or may affect active domains by means of effects on the tertiary structure (50). However, Shigemoto and cols. (59) have shown that this variant has no significant effect on dsRNA binding to IFIH1 or on IFN activation. The rs3747517 polymorphism replaces histidine to arginine in codon 843 of exon 13, within the MPH1 domain of the protein, which is conserved in ERCC4-like helicases and comprises two functional subdomains (helicase and C-terminal). Liu and cols. (51) suggest that the rs1990760

Table 1. Studies of polymorphisms in the *IFIH1* gene and type 1 *diabetes mellitus* (T1DM)

Polymorphism	Population and design	Results	Reference
rs1990760	United Kingdom (4,353 cases and 5,842 controls)	Association between the G allele and T1DM (OR = 0.86; P < 0.001)	(50)
rs1990760	Canada (7,721 T1DM patients and 9,679 controls + 2,214 nuclear family trios)	Association with risk for T1DM ($P = 4.1x10^{-5}$)	(54)
rs1990760	757/509 Hungarian/Finnish childhood-onset T1DM cases; 499/250 Hungarian/Finnish controls; and 529/924 Hungarian/Finnish nuclear family trios	In the Hungarian dataset, the A allele was more frequent among cases than among controls (OR = 1.29; P = 0.002). No association was observed in the Finnish dataset. The A allele was significantly overtransmitted in both family trio datasets	(52)
rs1990760 / rs2111485	Spain (311 T1DM patients and 535 non-diabetic controls)	No significant association with T1DM. The rs1990760 polymorphism showed only a trend towards association with T1DM (OR = 0.85, P = 0.07, for the G allele)	(57)
rs1990760	Belgium (1,981 T1DM patients, 2,092 non-diabetic controls and 430 case parent trios)	No significant association with T1DM	(56)
rs1990760 / rs35744605 / rs2111485 / rs13422767	U.S. (Georgia population: 1,434 T1DM patients and 1865 non-diabetic controls; Denver population: 612 T1DM patients and 552 controls)	Association of the major alleles of the four polymorphisms with T1DM (OR = 1.7 – 1.9; P < 0.001)	(51)
rs1990760 / rs3747517 / rs2111485	589 French-Canadian nuclear family trios	Association between allele A of the rs2111485 polymorphism and T1DM (OR = 0.84, P < 0.05). The rs1990760 and rs3747517 polymorphisms showed only a trend towards association with T1DM (P > 0.05)	(53)
rs35667974 / rs35337543 / rs35732034 / rs35744605 / rs1990760	United Kingdom (7,853 cases and 9,166 controls)	All five polymorphisms were associated with T1DM (OR 0.51 – 0.74; $P = 1.3 \times 10^{-3} – 2.1 \times 10^{-16}$)	(29)
rs1990760 / rs3747517	Han Chinese (464 T1DM patients and 465 controls)	Association between the rs3747517 polymorphism and T1DM (P < 0.001). No association between the rs1990760 and T1DM	(55)
rs6432714 / rs10930046	Northeast Brazil (196 T1DM patients and 176 healthy controls)	No significant association with T1DM	(58)

polymorphism may affect the sequence of the HNF-3b transcription factor binding site in the *IFIH1* gene, whereas the rs3747517 polymorphism may alter the binding site for the AP-1 transcription factor. As both polymorphisms are located 45-50kb from the start codon, it has yet to be determined whether these variants really play a role in the regulation of *IFIH1* expression or are merely in linkage disequilibrium with a functional polymorphism in this region (42,51).

The rs13422767 and rs2111485 polymorphisms are located within the 3' intergenic region adjacent to the *IFIH1* gene, 23kb and 13kb from the end of the 3'-UTR of the gene respectively. These polymorphisms do not change any known transcription factor binding sites, and it is not known whether they contribute to the regulation of *IFIH1* gene expression (51). Rare variants, however, are predicted to have significant biological effects on the *IFIH1* gene, whether by resulting in a truncated protein product by generating a stop codon in exon 10 (rs35744605), affecting splicing positions (rs35337543 and rs35732034, position +1 of introns 8 and 14 respectively), or changing a highly conserved amino acid (rs35667974 in exon 14) (29).

Bonifacio and cols. (60) investigated potential associations between cesarean delivery, islet autoimmunity, and genes involved in T1DM susceptibility in 1,650 German children born of one parent with T1DM and followed from birth to onset of anti-islet autoantibodies or diagnosis of T1DM (BABYDIAB study). Children delivered via cesarean section had a twofold higher risk of T1DM as compared with children born by vaginal delivered (hazard ratio = 2.5; P = 0.001). Furthermore, cesarean delivery was associated with faster progression to T1DM after onset of autoimmunity (P = 0.015). Interestingly, an increased risk of T1DM was only observed in children who were born by cesarean section and were homozygous for the G allele of *IFIH1* rs2111485 polymorphism (12-year risk of developing T1DM = 9.1 in G/G children delivered via cesarean section *vs.* 3% in G/G children born by vaginal delivered; P = 0.0001).

In short, the above-cited studies suggest that more than one *IFIH1* polymorphism contributes to T1DM susceptibility in several populations. Additional research is required to ascertain the biological effects of these polymorphisms.

Associations between *IFIH1* and other autoimmune conditions

Autoimmune diseases are distinct clinical syndromes characterized by changes in normal immune responsiveness due to loss of tolerance to one or more host constituents (61). Furthermore, it is widely known that genetic factors play a substantial role in the pathogenesis of autoimmune conditions. Accordingly, studies of several loci related to the immune system, such as the *IFIH1* gene, have been conducted in an attempt to provide better understanding of the pathogenesis of these diseases (62).

Interestingly, some studies have shown strong associations between the rs1990760 polymorphism of the *IFIH1* gene and other autoimmune diseases, such as psoriasis (63,64), chronic periodontitis (63), polymyositis (65), multiple sclerosis (57,66), systemic lupus erythematosus (67), and Graves' disease (68).

These associations between *IFIH1* and autoimmune conditions unrelated to viral infection, such as Graves' disease, suggest that IFIH1 may also play an endogenous immunoregulatory role unrelated to its function as a viral receptor (68). Additional studies are needed to identify the role of *IFIH1* in the pathogenesis of these conditions.

CONCLUSIONS

The *IFIH1* gene plays a major role in the innate immune response triggered by viral infection. Binding of viral replication-derived dsRNA to IFIH1 triggers the release of proinflammatory cytokines by immune system cells. This local inflammation and activation of antiviral defenses aims at eradicating infection and trigger apoptosis of virus-infected cells. However, in some genetically susceptible individuals, this defense system fails to work properly, inducing excessive, progressive inflammation and prolonged death of beta-cells instead and, thus, predisposing them to the development of T1DM. Hence, *IFIH1* is a good candidate gene for T1DM. Indeed, several studies conducted in different populations suggest that more than one *IFIH1* polymorphism is associated with T1DM. The rs1990760 polymorphism has also been associated with other autoimmune conditions, such as Graves' disease and systemic lupus erythematosus. Additional studies are required to elucidate the molecular mechanisms underlying the association between these polymorphisms and T1DM and other autoimmune diseases. Knowledge of the factors associated with T1DM development will enable a keener understanding of its pathogenesis and may provide more effective approaches for the treatment and prevention of T1DM.

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