Enterovirus RNA in Peripheral Blood May Be Associated with the Variants of rs1990760, a Common Type 1 Diabetes Associated Polymorphism in IFIH1

Ondrej Cinek1*, German Tapia2, Elisabet Witsø2, Lenka Kramna1, Katerina Holkova1, Trond Rasmussen2, Lars C. Stene2, Kjersti S. Rønningen3

1 2nd Faculty of Medicine, Department of Paediatrics, Charles University in Prague and University Hospital Motol, Prague, The Czech Republic, 2 Division of Epidemiology, Norwegian Institute of Public Health, Oslo, Norway, 3 Oslo University Hospital, Rikshospitalet, Oslo, Norway

Abstract

Objective: Polymorphisms in the IFIH1 (common rs1990760 and four rare rs35667974, rs35337543, rs35744605, rs35732034) have been convincingly associated with type 1 diabetes. The encoded protein (interferon-induced helicase C domain-containing protein 1) senses double-stranded RNA during replication of Picornavirales, including Enterovirus, a genus suspected in the etiology of type 1 diabetes. We therefore investigated whether the polymorphisms are associated with differences in the frequency of enterovirus RNA in blood.

Research Design and Methods: The study included 1001 blood samples, each from a child participating in the Norwegian ‘Environmental Triggers of Type 1 Diabetes: the MIDIA study’. The enterovirus RNA was tested using qualitative semi-nested real-time reverse transcriptase PCR on RNA extracted from frozen cell packs after removal of plasma. Stool samples previously analyzed for enterovirus RNA were available in 417 children.

Results: The genotypes of IFIH1 rs1990760 were associated with different frequencies of enterovirus RNA in blood (7.0%, 14.4% and 9.5% bloods were enterovirus positive among children carrying the Ala/Ala, Ala/Thr and Thr/Thr genotypes, respectively, $p = 0.012$). This association remained essentially unchanged after adjustment for age and calendar year. The presence of enterovirus in the concomitantly sampled stool further increased the likelihood of enterovirus RNA in blood (odds ratio 2.40, CI 95% 1.13–4.70), but did not affect the association with IFIH1 rs1990760. The rare polymorphisms (individually, or pooled) were not significantly associated with enterovirus RNA in blood.

Conclusions: The common IFIH1 SNP may modify the frequency of enterovirus RNA in blood of healthy children. This effect can help explain the association of T1D with type 1 diabetes.

Introduction

Enteroviruses, namely the members of the species Human enterovirus A - D (genus Enterovirus, family Picornaviridae, order Picornavirales), have been suspected from involvement in the etiology and pathogenesis of type 1 diabetes (T1D), reviewed e.g. in [1]. Enterovirus positivity has been observed in the pancreatic tissues and gut mucosa at T1D onset more often than in matched controls [2–4], although its presence at disease onset may be a consequence rather than a cause of the autoimmune process. Reports are inconclusive as to the actual ability of enterovirus to initiate the autoimmune process leading to T1D: enterovirus can predict a small fraction of the cases with autoimmune process in Finnish studies [5–8], while no association with autoimmune was found in other populations [9,10].

Recently, substantial support to the role of enterovirus in T1D pathogenesis was provided by the discovery of an association between the IFIH1 polymorphisms and T1D. The IFIH1 gene encodes for the interferon-induced helicase C domain-containing protein 1 which acts as a sensor of long intracellular double stranded RNA produced during replication of certain viruses, including Picornaviridae [11,12]. It is essential for activating antiviral responses, in particular the type I interferons (reviewed in [13]). Its association with T1D was first observed in a genome-wide scan where the common SNP rs1990760 had its minor allele (guanine, encoding the amino acid alanine at the position 946) associated with a slightly decreased risk of T1D as compared to the major allele (adenine, encoding the amino acid threonine at the position 946) associated with a slightly decreased risk of T1D as compared to the major allele (adenine, encoding the amino acid threonine at the position 946). It was then confirmed in several studies, and similar association has been detected also in other autoimmune disorders (e.g. multiple sclerosis...
[15,16] and Graves’ disease [17]). The causal role of IFIH1 was later reinforced by the discovery of four of its rare variants associated with T1D [18].

The mechanisms by which the IFIH1 polymorphisms modify the risk of T1D and other autoimmune diseases are presently unknown, but most works agree that the minor alleles (protective from T1D) are associated with diminished transcription or reduced function of the IFIH1 [19–22]. Conceivably, the IFIH1 polymorphisms might influence not only the intensity of inflammation following enterovirus infection, but also the frequency and timing of enterovirus infection, and thus modify the likelihood of prediabetic autoimmunity. This is a testable hypothesis: a weaker immune response to enterovirus (associated with the minor alleles) should be mirrored by increased replication of enterovirus, detectable either as its higher frequency, or higher viral load. In our earlier study where we studied enterovirus in faeces, one of the rare variants (rs35732034, a mutation in the conserved splice site of exon 14) was weakly associated with enterovirus in the faeces [23].

In the present study, we aimed to investigate whether the IFIH1 polymorphisms are related to the frequency of enterovirus RNA in blood samples obtained from healthy children. We expected that the findings in blood, as compared to our previous studies of gut infection, should better reflect the putative effect of IFIH1 on the course of infection: positivity for enterovirus in blood means that the virus has passed through immunological barriers, including the ones where IFIH1 acts.

Materials and Methods

Study subjects

The subjects were healthy children from the MIDIA study (a Norwegian acronym for “Environmental Triggers of Type 1 Diabetes”). Babies were recruited from the general Norwegian population at public health centers throughout the country [24]. During 2001–2007, 46,939 newborns were genotyped to identify HLA DRB1*04:01-DQA1*03-DQB1*03:02/DRB1*03-DQA1*05-DQB1*02 (DR4-DQ8/DR3-DQ2), the HLA genotype conferring the highest risk of T1D. The resulting high-risk cohort has been followed for development of autoimmunity and T1D. Furthermore, several hundred children with other genotypes were included in order to investigate the effect of HLA on the frequency of enterovirus infection in the gut [25].

In the current analysis, one blood sample and up to two concomitantly obtained stool samples were analyzed from each of 1,001 subjects (512 females, 489 males); 646 carrying the highest-risk genotype and 355 carrying other HLA genotypes. The median age at the blood sample collection was 12.3 months (IQR 9.2–13.5 months); the samples were taken over the period of 2004–2009, most often in 2005, 2006 and 2007 (27%, 34% and 20% respectively).

Ethics Statement

The parents consented in writing on behalf of the participants involved in the study (babies and infants recruited shortly after birth). The study, including the consent procedure, was approved by The Regional Committee for Medical Research Ethics (IRB name: “Regional Med Resch Ethics Comm South IRB #2 - South-East A”, IRB00001871) and the Norwegian Data Inspectorate.

Blood sample handling and nucleic acid extraction

Capillary blood was taken into an EDTA-anticoagulated tube (0.3–1 ml), centrifuged and plasma transferred to another tube, while the cell pack was stored frozen in the original tube at −80°C until further processing. From the cell sediments, DNA and RNA were co-purified using a modified procedure for the QIAamp DNA Blood BioRobot MDx Kit (Qiagen, Hilden, Germany). The cell pack was thawed in a refrigerated shaking incubator at +4°C, 300 rpm, adjusted with refrigerated normal saline to approximate 300 μl and added to 50 μl of Qiagen Protease prefiltered in a deep-well plate kept on ice. Then, 300 μl of Qiagen AL buffer was added and mixed and the extraction proceeded further according to the manufacturer’s instructions. Negative control (normal saline with diluted West Nile Virus Armored RNA, Asuragen, USA) was present in 7–9 positions in every 96-well plate, scattered in irregular patterns. The quantity of DNA in the extract was checked by determining the copy number of human ALB gene, whereas the cellular RNA content was assessed using reverse transcription real-time PCR for the human B2M or GUSB transcripts. Of the 1001 extracts, 25 had a very low or no detectable level of human mRNAs, which may have reflected a low cell content of the starting material caused by too small amount of the capillary sample, or a failure of the extraction process.

Detection of enterovirus RNA in nucleic acid from blood

Enterovirus RNA was tested using a semi-nested assay. The first round was performed as a one-tube reverse transcription PCR with the QuantiTect Probe RT-PCR kit (Qiagen) in a duplicate at a total volume of 15 μl, consisting of 1× QuantiTect PCR Master Mix, 900 nM ENT-R (5’-ATTGTCAACCATAGGACACAAGC-3’), 300 nM ENT-F (5’-CCCTGAATGGCGGTATAATCC-3’) primers, 150 nM ENT-probe (FAM-5’-AACCGAGTACCTTTGGGTGTCGGTGTTC-3’-TAMRA; the primers are probes in the first round were taken from [26]), 1× QuantiTect RT Mix and 2 μl template nucleic acid extract. The PCR run for 45 cycles with a thermal profile according to the manufacturer’s instructions. Two μl PCR product from each replicate were then mixed into 800 μl sterile PCR water in a deep-well plate and used as a template in the second PCR round. This second round was done using the AmpliTaq Gold chemistry (Applied Biosystems, Foster City, CA) in a total volume of 15 μl containing 1× PCR buffer, 200 nM each dNTP, 500 nM of each primer ENT-PolF (5’-GGTACCGGCGACAGTCTGTTG-3’), ENT-nonP (5’-GTCGTAATGGGAACACTGCGG-3’), ENT-R, 150 nM ENT-probe, 0.4 U AmpliTaq Gold polymerase and 2 μl of the diluted first-round product as a template. The thermal profile had 25 cycles. Along with the samples, a standard curve was run, constructed using serial six-fold dilutions of Armored RNA Enterovirus (Ambion, USA); the standard curve (spanning 375000 copies to a theoretical 1 copy per μl template) controlled the PCR efficiency in the first round, but lost its quantitative character in the second round because its first points had reached the PCR plateau already in the first round. Both rounds were run on a LightCycler 480 (Roche, Mannheim, Germany) equipped with a 384-well thermal block. Neither of the negative controls employed in the two rounds of PCR showed any signal. To further safeguard against cross-contamination, we verified that the enterovirus PCR products were diverse by using separate amplification in the second round with the ENT-PolF or ENT-nonP primers, and we attempted sequencing of these short products with the BigDye terminator 1.1 (Applied Biosystems, USA): which also showed a certain degree of diversity.

Stool samples

Stool samples were collected from all individuals in the study. However, some of the analyzed blood samples were collected...
beyond the date of the last available stool sample and some stools have not been yet tested. Therefore data on enterovirus in the concomitantly collected stool samples (time window spanning 30 days before to 15 days after collection of blood sample) are available only in a subset of 417 children. Of these 417 subjects who had at least one such sample available, 322 had one and 95 had two samples tested within the time window. Enterovirus RNA quantities in the stool samples were assayed previously using a single tube reverse transcription PCR [23,25].

**IFIH1 genotyping**

We genotyped the common IFIH1 SNP rs1990760 polymorphism (exon 15, A946T) using a TaqMan SNP assay (Applied Biosystems) and the minor IFIH1 polymorphisms (rs35667974, rs35337543, rs35744605, rs35732034) with the MassARRAY system using iPLEX chemistry (Sequenom, San Diego, CA), as reported previously [23]. All genotypes were in Hardy-Weinberg equilibrium. The genotype frequencies found for the common IFIH1 rs1990760 polymorphism were Ala/Ala 15.7%, Ala/Thr 48.5%, Thr/Thr 35.8%; the minor allele frequencies of rs1990760 polymorphism were Ala/Ala 15.7%, Ala/Thr 48.5%, Thr/Thr 35.8%, the minor allele frequencies of rs35667974, rs35337543, rs35744605 and rs35732034 were 2.9%, 0.6%, 0.7% and 0.6%, respectively. All carriers of the rare variants were heterozygous, neither carried more than one rare variant, and in total 9.6% of the subjects carried at least one minor allele of these four rare polymorphisms. After we detected an association of rs1990760 with enterovirus in blood, we further genotyped the rs3747517 (exon 13, H843R) using a TaqMan SNP assay.

**Statistical analysis**

The frequency of enterovirus RNA in blood was first assessed in univariate analyses against IFIH1 genotypes, gender, year of sampling, seasonality, age at blood sample, HLA risk of T1D and concomitant stool positivity for enterovirus. The variables associated therein were then entered into a multivariable regression model along with the level of HLA risk (highest T1D risk versus all other genotypes; the reason was its role in the study design, because the HLA risk was connected to a difference in the length of observation with stool samples, although by itself it was not associated with the risk of enterovirus RNA in blood). The dependent variable was dichotomous: blood positive or negative in the seminested enterovirus PCR. Two models were employed: the first included all blood samples but lacked the stool enterovirus as the independent variable; the second had concomitant stool positivity as one of the predictors and thus was limited to the 417 subjects where such stool samples were available.

A sensitivity analysis was performed for stool positivity as the predictor: the original time span of 30 days before to 15 days after the blood samples was extended to 60 days and then 90 days. Also, we assessed the effect of excluding 25 samples with little or no detectable human RNA, and of 12 children who developed islet autoimmunity.
We did not find any association between the four rare T1D-associated IFIH1 variants and the enterovirus RNA in blood, which is potentially attributable also to a lower statistical power due to the low frequency of these alleles.

Potential functional explanations
Several possible explanations exist for an observation of molecular heterosis [28]. An effect of the rs1990760 on transcription levels seems unlikely: a study utilizing an accurate and sensitive technology was unable to find differences in expression between its two alleles [39], and the authors proposed that the common IFIH1 polymorphisms may have other unknown functions, or act through changes in the tertiary structure of the protein. Since the IFIH1 protein homodimerizes and oligomerizes upon its binding to dsRNA [31], and as the rs1990760 encodes an amino acid in a region important for dimerization of the RIG-I-like proteins [32], we may speculate that a dimer consisting of two different protein variants may assemble less readily or act less effectively as compared to homodimers consisting of identical amino acids.

### Table 1. Factors tested for association with enterovirus positivity in the univariate analyses.

|                              | Enterovirus RNA in blood, frequency | Odds ratio [95% CI] for enterovirus RNA in blood (univariate analysis) |
|------------------------------|------------------------------------|-----------------------------------------------------------------------|
| **All subjects**             | 11.5% (115/1001)                   |                                                                       |
| **IFIH1 rs1990760** (1)      |                                    |                                                                       |
| subjects with Ala/Ala genotype | 7.01% (11/157)                     | 1.00: reference                                                       |
| subjects with Ala/Thr genotype | 14.4% (70/486)                     | 2.23 [1.15–4.33], p = 0.018                                          |
| subjects with Thr/Thr genotype | 9.50% (34/358)                     | 1.39 [0.69–2.83], p = 0.359                                          |
| **Rare variants in the IFIH1** (2) |                                 |                                                                       |
| subjects with wild-type variants at all four SNPs | 11.4% (103/903) | 1.00: reference                                                       |
| subjects with the rare variant positive at any of the SNPs | 12.5% (12/96) | 1.11 [0.59–2.10], p = 0.750                                          |
| **Gender**                   |                                    |                                                                       |
| Female                       | 11.5% (59/512)                     | 1.00: reference                                                       |
| Male                         | 11.5% (56/489)                     | 0.99 [0.67–1.46], p = 0.972                                          |
| **Seasonality**              |                                    |                                                                       |
| Month of sample, Walter and Elwood test: p = 0.41 |                                                                 |                                                                       |
| Jan–Mar                      | 9.1% (24/264)                      |                                                                       |
| Apr–Jun                      | 13.3% (37/278)                     |                                                                       |
| Jul–Sep                      | 12.1% (29/240)                     |                                                                       |
| Oct–Dec                      | 11.4% (25/219)                     |                                                                       |
| **Year of sampling** (3)     |                                    |                                                                       |
| 2004–2005                    | 15.3% (52/340)                     | 1.00: reference                                                       |
| 2006                         | 11.5% (39/340)                     | 0.72 [0.45–1.12], p = 0.144                                          |
| 2007–2009                    | 7.5% (24/321)                      | 0.45 [0.27–0.75], p = 0.002                                          |
| **Age at blood sample**      |                                    |                                                                       |
| 0–5.9 months                 | 19.5% (23/118)                     | 1.00: reference                                                       |
| 6–11.9 months                | 10.4% (26/251)                     | 0.48 [0.26–0.88], p = 0.018                                          |
| 12–17.9 months               | 13.4% (57/424)                     | 0.64 [0.38–1.09], p = 0.103                                          |
| 18+ months                   | 4.3% (9/208)                       | 0.19 [0.08–0.42], p<10^-3                                           |
| **HLA risk for T1D** (subset aged 10–14 mo) (4) | |                                                                       |
| high-risk                    | 12.5% (16/128)                     | 1.00: reference                                                       |
| children with other genotypes | 15.3% (54/353)                     | 1.26 [0.69–2.30], p = 0.443                                          |
| **Enterovirus positivity in stool taken within 30 days before to 15 days after the blood sampling date** (5) | none of the concomitant stool samples positive 13.5% (47/349) | 1.00: reference |
| none of the concomitant stool samples positive 13.5% (47/349) | 1.00: reference |
| one or more concomitant stool samples positive 25.0% (17/68) | 2.14 [1.14–4.02], p = 0.018 |

(1) IFIH1 946Ala is associated with a decrease, whereas the 946Thr with an increase in T1D risk in most studies [37]. Having detected the association of rs1990760 heterozygosity with an increased risk of enterovirus in blood, we further complemented the genotyping with the rs3747517 SNP which is in linkage disequilibrium with the rs1990760 (D’ = 1.0, r2 = 0.59 in our study, calculated according to [38]); however, the rs3747517 polymorphism was not significantly associated with enterovirus positivity (P = 0.063), and did not improve the fit of the multivariable analyses.
(2) The tested SNPs were rs35667974, rs35337543, rs35744605, rs35732034. Their minor alleles have been found associated with a decreased risk of T1D [18].
(3) The proportion of IFIH1 genotypes was not different among the years of sampling (p = 0.43).
(4) The analysis of HLA was restricted to a subset of sample taken at age 10–14 months, as the sampling schedules differed profoundly between the high-risk carriers and the non high-risk individuals. Whilst the former had a more even distribution of samples, the latter was almost exclusively restricted to the age 10–14 months and taken 2005–2006 only. High risk, the genotype DRB1*04:01-DQA1*03-DQB1*03:02/DRB1*03-DQA1*05-DQB1*02 (DR4-DQ8/DR3-DQ2).
(5) Stools from intervals more distant to the blood sample were not associated (the lowest p = 0.12 for interval 31 to 60 days before blood).

doi:10.1371/journal.pone.0048409.t001

We did not find any association between the four rare T1D-associated IFIH1 variants and the enterovirus RNA in blood, which is potentially attributable also to a lower statistical power due to the low frequency of these alleles.
variants. Indeed, in dimeric proteins, such a mechanism is often proposed as an explanation of molecular heterosis [28].

Comparison to previous studies on IFIH1

Chistiakov et al [22] investigated the effects of two of the rare substitutions (rs35744605, rs35667974) on the enterovirus frequency in the Russian population: the minor allele of the latter SNP (an allele protective from T1D) was associated with a clear and significant decrease in the enterovirus-positive proportion of RNA serum in T1D patients – a relation also rather difficult to explain. The authors did not investigate the rs1990760. One more study marginally touched the topic of IFIH1 related risk of enterovirus RNA in blood, but the sample size was far too small for efficient association testing [33].

In our previous study performed on the fecal material, we did not observe associations of enterovirus with the rs1990760, whereas we noted a borderline-significant increase in enterovirus frequency in carriers of one of the rare loss-of-function polymorphisms, the rs35732034 [23]. The influence of the immunity (and thus of IFIH1) on enterovirus replicating in the gut may be indeed less pronounced than in the blood – it is assumed that the antibodies can prevent spreading of the infection from the gut, but cannot provide “sterilizing immunity” [34]. The difference in IFIH1 effect between blood and stool is in line also with the current evidence linking enterovirus to islet autoimmunity (or T1D) more strongly for enterovirus RNA detected in blood than for fecal enteroviral RNA [35].

Relation between stool and blood positivity for enterovirus

The stool positivity for enterovirus was connected to more than two-fold increase in the frequency of enterovirus in blood (examples of enterovirus in blood following gut infection are shown in Figure 1). This may be an indication that viraemia arose from the strain present in the gut, or a reflection of a general propensity towards enterovirus infection both in the gut and blood. Enterovirus genotyping would be required to answer the question on identity of the strains: this will be pursued further, but it should be noted that discrimination of the enterovirus genotype present in blood is technically extremely difficult and many obstacles have to be overcome before the low-quantity enterovirus RNA can be genotyped.

Interestingly, only 25% enterovirus-positive blood samples had their respective concomitant stool also positive. There may be several explanations; however, neither of them can be tested in the present dataset. Firstly, the primary replication of the virus may have taken place elsewhere than in the gut (e.g. tonsils, nasal cavity), or may have been too short to be covered by the monthly intervals between stool samples. Secondly, the presence of virus in blood can be an indication of viral persistence long after the primary replication. Lastly, the presence (and quantity) of virus RNA in blood may vary with time, so that a single sample can ascertain only a fraction of subjects who have the virus replicating in their organs.

Table 2. The IFIH1-associated risk in a multivariable analysis.

| IFIH1 rs1990760 \( ^{(2)} \) | Odds ratio (95% CI), p, in a model without the stool positivity as predictor (1001 blood samples) | Odds ratio (95% CI), p, in a subset of samples where a concomitant stool was available\( ^{(3)} \) (417 blood samples) |
|---|---|---|
| Ala/Ala | 1.00, reference | 1.00, reference |
| Ala/Thr | 2.18 [1.12–4.27], p = 0.022 | 2.23 [0.93–5.32], p = 0.072 |
| Thr/Thr | 1.37 [0.67–2.82], p = 0.380 | 1.50 [0.59–3.79], p = 0.394 |

Stool 30 days before to 15 days after the blood

| Odds ratio (95% CI), p, in a subset of samples where a concomitant stool was available\( ^{(3)} \) (417 blood samples) |
|---|---|
| all negative | 1.00, reference |
| one or more positive | 2.40 [1.13–4.70], p = 0.010 |

Year of sampling

| Odds ratio (95% CI), p, in a subset of samples where a concomitant stool was available\( ^{(3)} \) (417 blood samples) |
|---|---|
| 2004–2005 | 1.00, reference |
| 2006 | 0.68 [0.43–1.07], p = 0.099 |
| 2007–2009 | 0.63 [0.34–1.12], p = 0.116 |

Age at blood sample

| Odds ratio (95% CI), p, in a subset of samples where a concomitant stool was available\( ^{(3)} \) (417 blood samples) |
|---|---|
| 0–5.9 months | 1.00, reference |
| 6–11.9 months | 0.44 [0.23–0.82], p = 0.011 |
| 12–17.9 months | 0.41 [0.20–0.84], p = 0.015 |
| 18+ months | 0.20 [0.09–0.45], p<10\(^{-3}\) |

HLA risk for T1D

| Odds ratio (95% CI), p, in a subset of samples where a concomitant stool was available\( ^{(3)} \) (417 blood samples) |
|---|---|
| high-risk\( ^{(3)} \) | 1.00, reference |
| other genotypes | 1.67 [0.88–3.15], p = 0.114 |

\( ^{(1)} \) A stool sample tested for enterovirus, collected within the interval from 30 days before to 15 days after the blood: the dataset was restricted to 417 bloods having at least one such tested stool available.

\( ^{(2)} \) Adding the terms for the rs3747517 did not improve the fit of the model.

\( ^{(3)} \) DRB1*04:01-DQA1*03-DQB1*03:02/DRB1*03-DQA1*05-DQB1*02 (DR4-DQ8/DR3-DQ2).

doi:10.1371/journal.pone.0048409.t002
Strength and weaknesses of the present work

The frequency of enterovirus in blood is considerably higher in our study as compared to what other groups observed in nondiabetic individuals [e.g. [5,6,33,36]], rendering our study higher power to detect differences among IFIH1 genotypes. The increased frequency may be a combination of epidemiological reasons (e.g. the age composition of the observed cohorts, difference between the Norwegian and other populations) and methodological aspects: in contrast to other studies, we used the blood cell pack as the source of nucleic acid - this may be an effective strategy given that the location of virus within the blood fractions has not been thoroughly studied in various phases of the infection. Interestingly, closely related Human parechovirus detected using a similar nested technique in a subset of the present blood samples was observed far less often (5/234 samples, 2.1%, 95% CI 0.94%–4.90%, data not shown), so that the possibility of epidemiological reasons still remains open.

A limitation with the current study is that each individual was represented by a single blood sample only. Also the relation between enterovirus RNA in blood and in stool could be better assessed with more frequent stool collection – yet our monthly sampling schedule is equally or more frequent as in other ongoing studies on diabetes (reviewed by [35]), being a compromise for the sake of the parents’ compliance. Future large scale studies investigating the potential relation between enterovirus RNA in the blood and risk of islet autoimmunity or T1D should probably include genotyping data for polymorphisms in the IFIH1 gene to hopefully arrive at a better understanding of the interrelationship among these factors.

Conclusion

We found that the common IFIH1 SNP rs1990760 was significantly associated with frequency of enterovirus RNA in blood of healthy children, and that this association was independent of enterovirus RNA in the faeces. This may help explain the mechanisms of IFIH1 association with T1D.
References

1. Tauriainen S, Oikarinen S, Oikarinen M, Hyoty H (2010) Enteroviruses in the pathogenesis of type 1 diabetes. Semin Immunopathol.

2. Oikarinen M, Tauriainen S, Honkanen T, Vissuri K, Karhumen P, et al. (2009) Analysis of pancreatic tissue in a child positive for islet cell antibodies. Diabetologia 51: 1796–1802.

3. Oikarinen M, Tauriainen S, Honkanen S, Honkanen T, Collin P, et al. (2012) Type 1 diabetes is associated with enterovirus infection in gut mucosa. Diabetes 61: 697–699.

4. Richardson SJ, Wilcock A, Bone AJ, Foulis AK, Morgan NG (2009) The prevalence of enteroviral capsid protein vpl immunostaining in pancreatic islets in human type 1 diabetes. Diabetologia 52: 1143–1151.

5. Lonnrot M, Salminen K, Knop M, Savola K, Kulmala P, et al. (2006) Enterovirus RNA in serum is a risk factor for beta-cell autoimmunity and clinical type 1 diabetes: a prospective study. Childhood Diabetes in Finland (DIME) Study Group. J Clin Endocrinol Metab 91: 1775–1783.

6. Oikarinen S, Martiskainen M, Tauriainen S, Huhtala H, Bonen J, et al. (2011) Enterovirus RNA in Blood Is Linked to the Development of Type 1 Diabetes. Diabetes 60: 276–279.

7. Sadelcha K, Hamalainen AM, Knip M, Lonnrot M, Koskela P, et al. (2003) Enterovirus infections as a risk factor for type 1 diabetes: virus analyses in a dietary intervention trial. Clin Exp Immunol 132: 271–277.

8. Salminen K, Sadelha K, Lonnrot M, Vahaulo P, Kulmala A, et al. (2003) Enterovirus infections are associated with the induction of beta-cell autoimmunity in a prospective birth cohort study. J Med Virol 69: 91–98.

9. Fuchtenbusch M, Irnstetter A, Jager G, Ziegler AG (2001) No evidence for an association of coxsackie-virus infections during pregnancy and early childhood with development of islet autoantibodies in offspring of mothers or fathers with type 1 diabetes. J Autoimmun 17: 333–340.

10. Graves PM, Rothart HA, Nix WA, Pallansch MA, Ertlh HA, et al. (2003) Prospective study of enteroviral infections and development of beta-cell autoimmunity. Diabetes autoimmunity study in the young (DAISY). Diabetes Res Clin Pract 59: 51–61.

11. Kato H, Takeuchi O, Sato S, Yoneyama M, Yamamoto M, et al. (2006) Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. Nature 441: 101–105.

12. Gitlin L, Barchet W, Gilfillan S, Cella M, Beutler B, et al. (2006) Essential role of MDA5: implications for resistance to type I diabetes. J Biol Chem 281: 13548–13554.

13. Chistiakov DA (2010) IFIH1, a gene implicated in antiviral responses, protect against type 1 diabetes. J Biol Chem 285: 13548–13554.

14. Smyth DJ, Cooper JD, Bailey R, Field S, Burren O, et al. (2006) A genome-wide association study of genetic variation at the IFIH1 locus in multiple sclerosis and polymorphisms of innate pattern recognition receptors TLR1-10, NOD1-2, DDX58, and IFIH1. J Neuroimmunol 212: 125–131.

15. Enevold C, Oturai AB, Sorensen PS, Ryder LP, Koch-Henriksen N, et al. (2009) Enterovirus RNA sequences in sera of schoolchildren in the general population. Nat Genet 38: 617–619.

16. Enevold C, Oturai AB, Sorensen PS, Ryder LP, Koch-Henriksen N, et al. (2009) Multiple sclerosis and polymorphisms of innate pattern recognition receptors TLR1-10, NOD1-2, DDX58, and IFIH1. J Neuroimmunol 212: 125–131.

17. Martinez A, Santiago JL, Ceut MC, de Las Heras V, de la Calle H, et al. (2008) IFIH1-GCA-KCNH7 locus: influence on multiple sclerosis risk. Eur J Hum Genet 16: 861–864.

18. Sutherland A, Davies J, Ower CJ, Vaikkakara S, Walker C, et al. (2007) Genomic polymorphisms at the interferon-induced helicase (IFIH1) locus contribute to Graves’ disease susceptibility. J Clin Endocrinol Metab 92: 3338–3341.

19. Nejentsev S, Walker N, Riches D, Egido M, Todd JA (2009) Rare variants of IFIH1, a gene implicated in antiviral responses, protect against type 1 diabetes. Science 324: 387–389.

20. Downes K, Pekalski M, Angus KL, Hardy M, Nutland S, et al. (2010) Reduced expression of IFIH1 is protective for type 1 diabetes. PLoS One 5: e11186.

21. Liu S, Wang H, Jia Y, Poddokie R, Liang Reddy MV, et al. (2008) The IFIH1 Gene Expression Are Significantly Associated with Type 1 Diabetes and IFIH1 Gene Expression in Peripheral Blood Mononuclear Cells. Hum Mol Genet.