Next-generation sequencing for viruses in children with rapid-onset type 1 diabetes

Lee, H-S; Briese, T.; Winkler, C.; Rewers, M.; Bonifacio, E.; Hyoty, H.; Pflueger, M.; Simell, O.; She, J. X.; Hagopian, W.; Lernmark, Åke; Akolkar, B.; Krischer, J. P.; Ziegler, A. G.

Published in:
Diabetologia

DOI:
10.1007/s00125-013-2924-y

2013

Brief report:

Next generation sequencing for viruses in children with rapid onset type 1 diabetes

Short running title: Viruses in type 1 diabetes

Hye-Seung Lee[1], Thomas Briese[2], Christiane Winkler[3], Marian Rewers[4], Ezio Bonifacio[5], Heikki Hyoty[6], Maren Pflüger[3], Olli Simell[7], Jin-Xiong She[8], Bill Hagopian[9], Ake Lernmark[10], Beena Akolkar[11], Jeff Krischer[1], Anette-G. Ziegler[3], and the TEDDY study

1. Pediatrics Epidemiology Center, Department of Pediatrics, University of South Florida, Tampa FL, USA

2. Center for Immunopathogenesis and Infectious Diseases, Columbia University, New York, USA

3. Institute of Diabetes Research, Helmholtz Zentrum München and Forschergruppe Diabetes e.V. Neuherberg, Germany

4. Barbara Davis Center for Childhood Diabetes, University of Colorado Denver, Aurora CO, U.S.A.

5. Center for Regenerative Therapies-Dresden, Dresden University of Technology, Dresden, Germany.

6. Department of Virology, University of Tampere, Finland

7. Department of Pediatrics, Turku University Central Hospital, Turku, Finland

8. Center for Biotechnology and Genomic Medicine, Medical College of Georgia, Georgia Regents University, Augusta GA, U.S

9. Pacific Northwest Diabetes Research Institute, Seattle WA, U.S.A.

10. Department of Clinical Sciences, Lund University, Malmo, Sweden

11. National Institute of Diabetes & Digestive & Kidney Disorders, Bethesda MD, U.S.A.

Corresponding author:

Prof. Dr. Anette-G. Ziegler, MD

Forschergruppe Diabetes e.V.

Institute of Diabetes Research

Helmholtz Zentrum München

Ingolstädter Landstraße 1

85764 Neuherberg

Germany

Tel: +49-89-3187-3405

Fax.: +49-89-3187-3144

E-mail: anette-g.ziegler@helmholtz-muenchen.de

Key words: TEDDY study, Infections, Islet autoimmunity, Type 1 diabetes mellitus

Word count: needs 2000 (current is 2001), Table: 3, Figures 2

Abstract

Viruses are candidate causal agents in the pathogenesis of type 1 diabetes. We hypothesized that children with a rapid onset of type 1 diabetes may have been exposed to strong causal infectious agents shortly before the initiation of islet autoimmunity and could be particularly helpful for the identification of causal agents of autoimmune diabetes. We used next generation sequencing to search for viruses in plasma samples and examined history of infections and fever in children from The Environmental Determinants of Diabetes in the Young (TEDDY) study who progressed to type 1 diabetes within 6 months from the appearance of islet autoimmunity, and in matched islet autoantibody negative controls. Viruses were infrequently detected in the period surrounding seroconversion in cases as well as controls. Moreover, infection history was similar between the children with rapid onset type 1 diabetes and control children. Episodes of fever were decreased in children with rapid onset diabetes. These findings do not support a major role for viruses in the pathogenesis of early rapid onset autoimmune diabetes in childhood.

Type 1 diabetes is an autoimmune disorder where disease onset is preceded by a pre-clinical period of islet autoimmunity (1). Check points in the pre-clinical pathogenesis include the initiation of islet autoimmunity, and progression to type 1 diabetes, and the rate of this process is variable (2). Although the majority of children who develop type 1 diabetes take years to progress from islet autoimmunity to diabetes, some children have a rapid or fulminant disease progression where clinical diabetes occurs within months from the appearance of islet autoantibodies. These cases appear to have uncontrolled beta cell destruction and could be particularly helpful for the identification of causal agents of autoimmune diabetes. We hypothesized that such children may have been exposed to strong causal agents shortly before the appearance of islet autoantibodies. Infections in infancy are candidate causal agents of islet autoimmunity and type 1 diabetes (3-5). We therefore examined history of documented infections and fever as well as plasma samples for virus by unbiased deep sequencing in the period surrounding the initiation of islet autoimmunity in children who transitioned rapidly between autoimmunity and disease in The Environmental Determinants of Diabetes in the Young (TEDDY) study (6).

Methods

The Environmental Determinants of Diabetes in the Young (TEDDY)

TEDDY is a prospective cohort study with the primary objective of identifying environmental factors that are associated with increased risk of islet autoimmunity and type 1 diabetes. It includes six clinical research centers in the US (Colorado, Georgia/Florida, Washington) and Europe (Finland, Germany, and Sweden). Infants were eligible if they were younger than 4.5 months and had high-risk human leukocyte antigen alleles (*HLA-DR, DQ*). From 2004-2010, TEDDY screened over 420,000 newborns and identified 21,589 children with high-risk HLA-DR, DQ genotypes. Of those, 8,677 (932 with first-degree family history of type 1 diabetes) were enrolled into prospective follow-up. Participants were seen every 3 months up to 4 years of age, and every 6 months after age 4 years. Blood samples were collected at each visit. Written informed consent was obtained from the parents. The Study was approved by the ethical committee of each site (6).

Study outcomes

The first primary outcome was the appearance of confirmed persistent islet autoimmunity, defined as positive for at least one autoantibody (GAD65A, IA-2A, or IAA) in both TEDDY core laboratories (Barbara Davis Center, Aurora, Colorado and the University of Bristol Laboratory, Bristol, UK) in two consecutive samples or in one sample in children who developed diabetes before a follow-up sample was available for autoantibody testing (7). Families were notified of the child's autoantibody results at their next study visit (i.e. after 3 months). The study endpoint was the development of type 1 diabetes as defined by the American Diabetes Association criteria (8).

Study subject and study design

Of the 8,677 enrolled children, 355 had islet autoimmunity, and 86 of these progressed to type 1 diabetes by July 2011 when this analysis was designed. Twenty-four of the 86 children with progression to type 1 diabetes developed type 1 diabetes within 6 months from the appearance of islet autoimmunity and were defined as rapidly progressed type 1 diabetes cases (Figure 1 A).

Sequencing study. This study investigated whether virus sequences were detectable in plasma samples at two time points: 1. the '*last islet autoantibody negative sample*' prior to islet auto-antibody seroconversion, and 2. the '*seroconversion sample'* (Figure 1B). Fourteen of the 24 cases had samples with sufficient volume available at both time points. For each of these 14 cases, 1 control child was selected. Controls were children who participated in the TEDDY study but remained negative for all three diabetes associated islet autoantibodies and for type 1 diabetes for at least 12 months after the respective event in cases. Controls were matched by clinical sites where the case child was enrolled and the family history of type 1 diabetes (yes or no), and were selected to have available plasma sample at the respective time points (Table 1). Controls were randomly selected from the pool of potential controls after being matched and conditioned.

TEDDY book study: This study investigated whether infections or fever were associated with early rapid progression to type 1 diabetes. All 24 cases, and for each case 3 controls were examined. Controls were selected by the same procedure as for the sequencing study. Three time points were examined in the 96 children (Figure 1B): 1. *Autoantibody negative period,* from birth to the last islet autoantibody negative sample, 2. *Seroconversion period*, from the last islet autoantibody negative sample to the first islet autoantibody positive sample, and 3. *Progression period*: from the first islet autoantibody positive sample to the date of type 1 diabetes diagnosis.

Nucleic acid sequencing

Total nucleic acids were extracted from 250 μl plasma samples. Nucleic acid quantity and quality were assessed (Bioanalyzer) and samples were prepared for unbiased RT-PCR amplification using random octamer primer pools optimized for amplification of non-human sequences, and linked to an arbitrary 17-mer primer sequence. cDNA was then randomly amplified by PCR with the octamer-linked 17-mer primer in conjunction with the 17-mer primer sequence without the random octamer tail in a 1:9 ratio (9). Products of >70 bp were selected by column purification (MinElute, Qiagen, Hilden, Germany) and ligated to barcoded linkers for sequencing on the 454 Genome Sequencer FLX (454 Life Sciences, Branford, CT, USA) without fragmentation of the products (10). Raw sequence reads were trimmed to remove sequences derived from amplification primers and linkers, and filtered to eliminate highly repetitive sequences. Sequences were then clustered into non-redundant sets and unique reads assembled into contiguous sequences, which were compared to the non-redundant GenBank database at the nucleotide and the translated amino acid level.

TEDDY book data

Acute infections or illnesses experienced between study visits were collected with the corresponding dates from interviewing parents and provided every 3 months starting from age 6 months. Acute infections or illnesses were recorded using the International Classification of Disease (ICD)-10 codes, and categorized as respiratory tract, gastrointestinal or other infection. Fever was documented using one of the following ICD-10 codes; R50, R50.9, R50.8 or R56, and was assessed when reported with or without infection. Fever with a measured temperature ≥38°C (101°F) was considered high fever.

Statistical analysis

Findings from the sequencing study were summarized. Overall infections and fever reports before type 1 diabetes onset were analyzed and then assessed according to the three periods of disease progression in the study design. Infections and fever reported during each period of interest were examined as the count of all reports or the indicator of any report. Number of observations with the proportion or the mean with standard deviation (SD) was reported depending on the type of data. Conditional logistic regression adjusted for maternal age at delivery was used to obtain the estimate of odds ratio of the factor of interest in comparison of the matched case and controls.

P-values less than 0.05 were used to indicate statistical significance. All reported *p*-values are two-sided without adjustment for multiple testing. All statistical analyses were performed using SAS 9.2 (SAS Institute, Cary, NC).

Results

Sequencing study

Unbiased sequencing identified viruses in 6 of 56 plasma samples (Table 1). Virus positive samples were from 4 (1 case, 3 control children) of the 28 children analysed. The human rhinovirus C was found in one case in the last islet autoantibody negative sample. This case did not experience fever and reported respiratory infections 3 times during the autoantibody negative period. Of the 3 control children with viruses in their plasma samples, one child had the GB virus C/Hepatitis virus G in both samples, one child had the Human Herpesvirus 6 in both samples, and one child had the human parvovirus B19 in the seroconversion sample. No virus sequences were identified in all other samples.

TEDDY book study

All cases and 70 of 72 controls reported at least one infection prior to the onset of type 1 diabetes (Table 2). A similar proportion of cases and controls reported infections in the islet autoantibody negative period (75% vs 74%), seroconversion period (71% vs 72%), and progression period (71% vs 67%). Moreover, the number of infections per child during each of these periods was similar between cases and controls (Table 3). Respiratory infections were the most commonly reported infections in both cases and controls.

In contrast to the reported infections, reported fever differed between the cases and controls (Table 2). Fever was reported less frequently in cases as compared to controls in the whole period to type 1 diabetes ($p=0.032$). Of interest, this difference was only seen in the seroconversion period ($p=0.030$) and progression period ($p=0.033$), and not seen in the autoantibody negative period. This difference appeared to be due to decreased episodes of fever during reported infections.

Discussion

Infectious agents are frequently, but inconclusively discussed candidate causal agents in the pathogenesis of type 1 diabetes (11-16). Here, using state-of-the-art methods for virus detection, we report that viruses are infrequently detected in the period surrounding seroconversion in children who progress to diabetes rapidly after developing islet autoimmunity. Moreover, children who developed type 1 diabetes rapidly in early childhood did not have more or different infections than matched islet autoantibody negative control children. Of potential interest, episodes of fever associated with infection were decreased in the children with rapid progression to diabetes. The findings do not support a prominent role for viruses in the pathogenesis of early rapid onset autoimmune diabetes in childhood.

The findings were generated on a subset of type 1 diabetes cases within the TEDDY study. For the infection history data, they represent all children in whom diabetes was diagnosed within 6 months of islet autoantibody in the study, and for the deep sequencing data, around 60% of such cases. In both analyses, controls from the TEDDY study that were well matched to cases were used as the comparator. Both children with and without a family history of type 1 diabetes were represented. Limitations include the relatively small numbers of cases studied, and the sampling which although is at 3 month intervals, may be too wide to identify virus.

Findings with respect to virus and infection history in type 1 diabetes have been controversial. Although the literature repeatedly reports associations between type 1 diabetes and viral infections (14-18), in particular enterovirus infections, there are also substantial reports that fail to confirm the findings (11-13). We had expected that the cases examined in this study would provide a best-case scenario for the identification of viruses if they were causal, but failed to detect virus in plasma from all but one of the 14 cases studied. We conclude either that a recent virus infection is not a frequent cause of type 1 diabetes in children with a rapid diabetes onset or that we lack the sensitivity to detect virus infection. Deep sequencing

techniques are currently state of the art and have been used to identify viral cause of disease (19). Within the cohort, viruses were consistently identified in both samples in 2 children suggesting that the methodology can reproducibly detect infection at 3 monthly intervals in plasma. Moreover, if we assume that virus is only present for 2 weeks, the deep sequencing technique is 100% sensitive, and sampling was random, then we would expect to be able to identify virus in around one quarter of actual cases where virus was present for two weeks. Under these assumptions, the one case identified would therefore correspond to less than half the cases analysed. The infection history data also does not support a prominent role of virus in these cases. Although infections were reported frequently in the children, their frequency was similar in cases and controls. Of potential interest, fever was infrequent in the cases, despite similar frequencies of infections in the cases and controls, suggesting that fever may provide protection in the disease pathogenesis and warrants further investigation.

In conclusion, in depth examination of cases of rapidly developing autoimmune diabetes in early childhood failed to find evidence for a viral cause of islet autoimmunity and progression to diabetes.

Conflict of interest statement: We declare that we have no conflict of interest.

Author Contributions

AZ, EB, and JK designed this project within the TEDDY study. HL reviewed the data and undertook statistical analysis, interpretation of the results, and contributed to the writing of the manuscript. TB performed virus sequencing and critically reviewed the manuscript for intellectual content. CW and MP acquired and reviewed the data and contributed to writing the manuscript and critically reviewed the manuscript for intellectual content. HH was involved in the interpretation of the results. AZ, MR, OS, JXS; BH and AK are principal investigator of the TEDDY study, designed the study, and acquired and reviewed the data,

and critically reviewed the manuscript for intellectual content. AZ and EB contributed to the statistical analysis, were involved in the interpretation of the results, and wrote the manuscript.

Acknowledgement:

Funded by DK 63829, 63861, 63821, 63865, 63863, 63836, 63790 and UC4DK095300 and Contract No. HHSN267200700014C from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institute of Allergy and Infectious Diseases (NIAID), National Institute of Child Health and Human Development (NICHD), National Institute of Environmental Health Sciences (NIEHS), Juvenile Diabetes Research Foundation (JDRF), and Centers for Disease Control and Prevention (CDC).

The Teddy Study Group

Colorado Clinical Center: Marian Rewers, M.D., Ph.D., PI[1,4,6,10,11], Katherine Barriga[12], Kimberly Bautista[12], Judith Baxter[9,12,15], George Eisenbarth, M.D., Ph.D., Nicole Frank[2], Patricia Gesualdo[2,6,12,14,15], Michelle Hoffman[12,13,14], Lisa Ide, Rachel Karban[12], Edwin Liu, M.D.[13], Jill Norris, Ph.D.[2,12], , Kathleen Waugh[7,12,15] Adela Samper-Imaz, Andrea Steck, M.D., University of Colorado, Anschutz Medical Campus, Barbara Davis Center for Childhood Diabetes.

Georgia/Florida Clinical Center: Jin-Xiong She, Ph.D.,PI[1,3,4,11], Desmond Schatz, M.D.*[4,5,7,8], Diane Hopkins[12], Leigh Steed[12,13,14,15], Jamie Thomas*[6,12], Katherine Silvis[2], Michael Haller, M.D.*[14], Meena Shankar*[2], Kim English, Richard McIndoe, Ph.D., Haitao Liu, M.D.†, John Nechtman†, Ashok Sharma, Joshua Williams, Gabriela Foghis, Stephen W. Anderson, M.D.[^] Georgia Health Sciences University, *University of Florida, †Jinfiniti Biosciences LLC, Augusta, GA, [^]Pediatric Endocrine Associates, Atlanta, GA.

Germany Clinical Center: Anette G. Ziegler M.D.,PI[1,3,4,11], Andreas Beyerlein Ph.D.[2], Ezio Bonifacio Ph.D.*[5], Kristina Foterek[¥2], Anna Huppert, M.D., Lydia Henneberger[2,12], Michael Hummel M.D.[13], Sandra Hummel Ph.D.[2], Mathilde Kersting Ph.D.[¥2], Annette Knopff[7], Sibylle Koletzko, M.D.[¶13], Stephanie Krause, Claudia Peplow*[12], Maren Pflüger Ph.D.[6], Roswith Roth Ph.D.[9], Julia Schenkel, Joanna Stock, Elisabeth Strauss, Katharina Warncke M.D.[14], Christiane Winkler Ph.D.[2,12,15], , Forschergruppe Diabetes e.V. at Helmholtz Zentrum München, *Center for Regenerative Therapies, TU Dresden, [¶]Dr. von Hauner Children´s Hospital,Department of Gastroenterology, Ludwig Maximillians University Munich, [¥]Research Institute for Child Nutrition, Dortmund.

Finland Clinical Center: Olli G. Simell, M.D., Ph.D.,PI[¥^1,4,11,13], Heikki Hyöty, M.D., Ph.D.*[±6], Jorma Ilonen, M.D., Ph.D.[¥ ¶3], Mikael Knip, M.D., Ph.D.*[±], Maria Lonnrot, M.D., Ph.D.*[±6], Elina Mantymaki[¥^], Juha Mykkänen, Ph.D.[^¥ 3], Kirsti Nanto-Salonen, M.D., Ph.D.[¥ ^12], Tiina Niininen[±]*, Mia Nyblom*[±], Anne Riikonen*[±2], Minna Romo[¥^], Barbara Simell[¥^12,15], Tuula Simell, Ph.D.[¥^9,12], Ville Simell[^¥13], Maija Sjöberg[¥^12,14], Aino Stenius[µ¤], Eeva Varjonen[¥^], Riitta Veijola, M.D., Ph.D. [µ¤], Suvi M. Virtanen, M.D., Ph.D.*[±§2]. [¥]University of Turku, *University of Tampere, [µ]University of Oulu, [^]Turku University Hospital, [±]Tampere University Hospital, [¤]Oulu University Hospital, §National Institute for Health and Welfare, Finland, [¶]University of Kuopio.

Sweden Clinical Center: Åke Lernmark, Ph.D.,PI[1,3,4,8,10,11,15], Daniel Agardh, M.D., Ph.D.[13], Peter Almgren, Eva Andersson, Carin Andrén-Aronsson[2,13], Maria Ask, Ulla-Marie Karlsson, Corrado Cilio, M.D.[5], Ph.D., Jenny Bremer, Emilie Ericson-Hallström, Thomas Gard, Joanna Gerardsson, Gertie Hansson[12,14], Monica Hansen, Susanne Hyberg, Rasmus Håkansson, Fredrik Johansen, Linda Jonsson, Helena Larsson M.D., Ph.D.[14], Barbro Lernmark, Ph.D.[9,12], Maria Markan, Theodosia Massadakis, Jessica Melin, Maria Månsson-Martinez, Anita Nilsson, Kobra Rahmati, Monica Sedig Järvirova, Sara Sibthorpe, Birgitta Sjöberg, Anna Skogberg, Carina Törn, Ph.D. [3,15], Anne Wallin, Åsa Wimar, Sofie Åberg. Lund University.

Washington Clinical Center: William A. Hagopian, M.D., Ph.D., PI[1,3,4, 5, 6,7,11,13, 14], Xiang Yan, M.D., Michael Killian[6,7,12,13], Claire Cowen Crouch[12,14,15], Kristen M. Hay[2], Stephen Ayres, Carissa Adams, Brandi Bratrude, David Coughlin, Greer Fowler, Czarina Franco, Carla Hammar, Diana Heaney, Patrick Marcus, Arlene Meyer, Denise Mulenga, Elizabeth Scott, Jennifer Skidmore, Joshua Stabbert, Viktoria Stepitova, Nancy Williams. Pacific Northwest Diabetes Research Institute.

Pennsylvania Satellite Center: Dorothy Becker, M.D., Margaret Franciscus[12], MaryEllen Dalmagro-Elias[2], Ashi Daftary, M.D. Children's Hospital of Pittsburgh of UPMC.

Data Coordinating Center: Jeffrey P. Krischer, Ph.D.,PI[1,4,5,10,11], Michael Abbondondolo, Lori Ballard[3,9,14,15], Rasheedah Brown[12,15], Brant Burkhardt, Ph.D.[5,6], David Cuthbertson, Christopher Eberhard, Steven Fiske, Veena Gowda, David Hadley, Ph.D.[3,13], Hye-Seung Lee, Ph.D.[3,6,13,15], Shu Liu, Kristian Lynch, Ph.D.[9], Jamie Malloy, Cristina McCarthy[12,15], Wendy McLeod[2,5,6,13,15], Laura Smith, Ph.D.[9], Susan Smith[12,15], Ulla Uusitalo, Ph.D.[2,15], Kendra Vehik, Ph.D. [4,5,9,14,15], Earnest Washington, Jimin Yang, Ph.D., R.D.[2,15]. University of South Florida.

Project scientist: Beena Akolkar, Ph.D.[1,3,4,5,7,10,11], National Institutes of Diabetes and Digestive and Kidney Diseases.

Other contributors: Kasia Bourcier[5], National Institutes of Allergy and Infectious Diseases, Ph.D. Thomas Briese, Ph.D.[6,15], Columbia University, Henry Erlich, Ph.D.[3], Children's Hospital Oakland Research Institute, Suzanne Bennett Johnson, Ph.D.[9,12], Florida State University, Steve Oberste, Ph.D.[6], Centers for Disease Control and Prevention.

Committees:

[1]Ancillary Studies, [2]Diet, [3]Genetics [4]Human Subjects/Publicity/Publications, [5]Immune Markers, [6]Infectious Agents, [7]Laboratory Implementation, [8]Maternal Studies, [9]Psychosocial, [10]Quality Assurance, [11]Steering, [12]Study Coordinators, [13]Celiac Disease, [14]Clinical Implementation, [15]Quality Assurance Subcommittee on Data Quality.

References:

1. Ziegler AG, Nepom GT. Prediction and pathogenesis in type 1 diabetes. Immunity. 2010;32(4):468-478.

2. Atkinson MA, Eisenbarth GS. Type 1 diabetes: new perspectives on disease pathogenesis and treatment. Lancet. 2001;358(9277):221-229.

3. Roivainen M, Klingel K. Virus infections and type 1 diabetes risk. Curr Diab Rep. 2010;10(5):350-356.

4. Tauriainen S, Oikarinen S, Oikarinen M, Hyoty H. Enteroviruses in the pathogenesis of type 1 diabetes. Semin Immunopathol. 2011;33(1):45-55.

5. Stene LC, Rewers M. Immunology in the clinic review series; focus on type 1 diabetes and viruses: the enterovirus link to type 1 diabetes: critical review of human studies. Clin Exp Immunol. 2012;168(1):12-23.

6. The Environmental Determinants of Diabetes in the Young (TEDDY) study: study design. Pediatr Diabetes. 2007;8(5):286-298.

7. Bonifacio E, Yu L, Williams AK, Eisenbarth GS, Bingley PJ, Marcovina SM, et al. Harmonization of glutamic acid decarboxylase and islet antigen-2 autoantibody assays for national institute of diabetes and digestive and kidney diseases consortia. J Clin Endocrinol Metab. 2010;95(7):3360-3367.

8. Puavilai G, Chanprasertyotin S, Sriphrapradaeng A. Diagnostic criteria for diabetes mellitus and other categories of glucose intolerance: 1997 criteria by the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (ADA), 1998 WHO consultation criteria, and 1985 WHO criteria. World Health Organization. Diabetes Res Clin Pract 1999; 44:21-26.

9. Quan PL, Palacios G, Jabado OJ, Conlan S, Hirschberg DL, Pozo F, Jack PJ, Cisterna D, Renwick N, Hui J, Drysdale A, Amos-Ritchie R, Baumeister E, Savy V, Lager KM, Richt JA, Boyle DB, García-Sastre A, Casas I, Perez-Breña P, Briese T, Lipkin WI. Detection of respiratory viruses and subtype identification of influenza A viruses by GreeneChipResp oligonucleotide microarray. J Clin Microbiol. 2007;45(8):2359-64.

10. Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, Berka J, Braverman MS, Chen YJ, Chen Z, Dewell SB, Du L, Fierro JM, Gomes XV, Godwin BC, He W, Helgesen S, Ho CH, Irzyk GP, Jando SC, Alenquer ML, Jarvie TP, Jirage KB, Kim JB, Knight JR, Lanza JR, Leamon JH, Lefkowitz SM, Lei M, Li J, Lohman KL, Lu H, Makhijani VB, McDade KE, McKenna MP, Myers EW, Nickerson E, Nobile JR, Plant R, Puc BP,

Ronan MT, Roth GT, Sarkis GJ, Simons JF, Simpson JW, Srinivasan M, Tartaro KR, Tomasz A, Vogt KA, Volkmer GA, Wang SH, Wang Y, Weiner MP, Yu P, Begley RF, Rothberg JM. Genome sequencing in microfabricated high-density picolitre reactors. Nature. 2005 Sep 15;437(7057):376-380.

11. Füchtenbusch M, Irnstetter A, Jäger G, Ziegler AG. 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. 2001;17(4):333-340.

12. Mercalli A, Lampasona V, Klingel K, Albarello L, Lombardoni C, Ekström J, Sordi V, Bolla A, Mariani A, Bzhalava D, Dillner J, Roivainen M, Bosi E, Piemonti L. No evidence of enteroviruses in the intestine of patients with type 1 diabetes. Diabetologia. 2012;55(9):2479-2488.

13. Simonen-Tikka ML, Pflueger M, Klemola P, Savolainen-Kopra C, Smura T, Hummel S, Kaijalainen S, Nuutila K, Natri O, Roivainen M, Ziegler AG. Human enterovirus infections in children at increased risk for type 1 diabetes: the Babydiet study. Diabetologia. 2011;54(12):2995-3002.

14. Oikarinen S, Martiskainen M, Tauriainen S, Huhtala H, Ilonen J, Veijola R, Simell O, Knip M, Hyöty H. Enterovirus RNA in blood is linked to the development of type 1 diabetes. Diabetes. 2011;60(1):276-279.

15. Stene LC, Oikarinen S, Hyöty H, Barriga KJ, Norris JM, Klingensmith G, Hutton JC, Erlich HA, Eisenbarth GS, Rewers M. Enterovirus infection and progression from islet autoimmunity to type 1 diabetes: the Diabetes and Autoimmunity Study in the Young (DAISY). Diabetes. 2010;59(12):3174-3180.

16. Salminen K, Sadeharju K, Lönnrot M, Vähäsalo P, Kupila A, Korhonen S, Ilonen J, Simell O, Knip M, Hyöty H. Enterovirus infections are associated with the induction of beta-cell autoimmunity in a prospective birth cohort study. J Med Virol. 2003;69(1):91-98.

17. Dotta F, Censini S, van Halteren AG, Marselli L, Masini M, Dionisi S, Mosca F, Boggi U, Muda AO, Prato SD, Elliott JF, Covacci A, Rappuoli R, Roep BO, Marchetti P. Coxsackie B4 virus infection of beta cells and natural killer cell insulitis in recent-onset type 1 diabetic patients. Proc Natl Acad Sci U S A. 2007;104(12):5115-5120.

18. Yoon JW, Austin M, Onodera T, Notkins AL. Isolation of a virus from the pancreas of a child with diabetic ketoacidosis. N Engl J Med. 1979;300(21):1173-1179.

19. Palacios G, Druce J, Du L, Tran T, Birch C, Briese T, Conlan S, Quan PL, Hui J, Marshall J, Simons JF, Egholm M, Paddock CD, Shieh WJ, Goldsmith CS, Zaki SR, Catton M, Lipkin

WI. A new arenavirus in a cluster of fatal transplant-associated diseases. N Engl J Med. 2008;358(10):991-998.

Table 1. Viruses detection in 56 plasma samples at the two time points in the sequencing study: 1st sample: the 'last islet autoantibody negative sample' prior to islet autoantibody seroconversion, and 2nd sample: the 'seroconversion sample' of 14 cases and controls pairs

Pair		Clinical Site*	family history of type 1 diabetes	Age at 1st sample (months)	Virus detection at 1st sample	Age at 2nd sample (months)	Virus detection at 2nd sample	Age at diagnosis of type 1 diabetes (months)
1	Case	FIN	no	19	NEG	22	NEG	27
1	Control	FIN	no	18	NEG	21	NEG	-
2	Case	COL	yes	9	Human rhinovirus C (HRVNEGC)	11	NEG	15
2	Control	COL	yes	9	NEG	12	Human parvovirus B19 (B19V)	-

3	Case	COL	no	17	NEG	21	NEG	21
3	Control	COL	no	18	NEG	21	NEG	-
4	Case	GER	yes	16	NEG	22	NEG	28
4	Control	GER	yes	19	NEG	22	NEG	-
5	Case	GER	yes	7	NEG	9	NEG	12
5	Control	GER	yes	6	NEG	10	NEG	-
6	Case	COL	no	15	NEG	18	NEG	20
6	Control	COL	no	14	NEG	18	NEG	-
7	Case	GER	yes	7	NEG	9	NEG	13
7	Control	GER	yes	6	NEG	10	NEG	-
8	Case	WAS	no	6	NEG	9	NEG	10
8	Control	WAS	no	5	NEG	8	NEG	-
9	Case	WAS	yes	24	NEG	27	NEG	33
9	Control	WAS	yes	24	NEG	27	NEG	-
10	Case	GER	no	7	NEG	10	NEG	12
10	Control	GER	no	6	NEG	9	NEG	-

11	Case	GEO	yes	6	NEG	9	NEG	14
11	Control	GEO	yes	7	Human herpesvirus 6 (HHVNEG6)	10	Human herpesvirus 6 (HHVNEG6)	-
12	Case	SWE	yes	6	NEG	9	NEG	15
12	Control	SWE	yes	6	NEG	8	NEG	-
13	Case	COL	no	9	NEG	12	NEG	18
13	Control	COL	no	8	NEG	12	NEG	-
14	Case	FIN	no	7	NEG	10	NEG	15
14	Control	FIN	no	6	GB virus C/ Hepatitis virus G (GBVNEGC)	9	GB virus C/ Hepatitis virus G (GBVNEGC)	-

* COL: Colorado, FIN: Finland, GER: Germany, GEO: Georgia/Florida, SWE: Sweden, WAS: Washington

NEG: negative

Table 2. Number of children who reported one or more infections and fever reports in the all time points investigated

		Case n (%)	Control n (%)	OR (95% CI)	p-value
Birth to Type 1 Diabetes	Any infection	24 (100%)	70 (97%)		
	Fever	**17 (71%)**	**62 (86%)**	**0.22 (0.06-0.88)**	**0.032**
	High fever†	15 (63%)	56 (78%)	0.42 (0.14-1.24)	0.116
	Fever without infectious illness	9 (38%)	22 (31%)	1.21 (0.44-3.30)	0.715
	Fever with any infectious illness	14 (58%)	56 (78%)	0.36 (0.12-1.10)	0.072
	Respiratory tract	24 (100%)	68 (94%)		
	Gastrointestinal tract	11(46%)	29 (40%)	1.55 (0.50-4.76)	0.447
	Other	5 (21%)	13 (18%)	1.40 (0.36-5.48)	0.630
Autoantibody negative period	Any infection	18 (75%)	53 (74%)	0.94 (0.20-4.52)	0.940
	Fever	13 (54%)	38 (53%)	1.10 (0.31-3.85)	0.882
	High fever†	12 (50%)	30 (42%)	1.54 (0.49-4.81)	0.463
	Fever without infectious illness	6 (25%)	8 (11%)	1.95 (0.59-6.50)	0.275
	Fever with any infectious illness	9 (38%)	33 (46%)	0.76 (0.22-2.60)	0.658
	Respiratory tract	18 (75%)	49 (68%)	1.89 (0.40-8.89)	0.420
	Gastrointestinal tract	5 (21%)	16 (22%)	0.96 (0.27-3.44)	0.944

	Other	2 (8%)	8 (11%)	0.57 (0.08-3.87)	0.564
	Any infection	17 (71%)	52 (72%)	0.95 (0.31-2.86)	0.922
	Fever	**4 (17%)**	**30 (42%)**	**0.27 (0.08-0.88)**	**0.030**
	High fever†	4 (17%)	25 (35%)	0.39 (0.12-1.23)	0.108
Seroconversion period	Fever without infectious illness	2 (8%)	11 (15%)	0.47 (0.09-2.56)	0.380
	Fever with any infectious illness	**3 (13%)**	**26 (36%)**	**0.23 (0.06-0.88)**	0.032
	Respiratory tract	15 (63%)	49 (68%)	0.71 (0.24-2.15)	0.543
	Gastrointestinal tract	3 (13%)	10 (14%)	1.25 (0.27-5.84)	0.776
	Other	1 (4%)	4 (6%)	1.11 (0.10-11.86)	0.931
	Any infection	17 (71%)	48 (67%)	1.34 (0.30-6.08)	0.706
	Fever	**5 (21%)**	**30 (42%)**	**0.14 (0.02-0.85)**	**0.033**
	High fever†	**3 (13%)**	**26 (36%)**	**0.11 (0.01-0.90)**	**0.040**
Progression period	Fever without infectious illness	2 (8%)	8 (11%)	0.76 (0.14-4.13)	0.752
	Fever with any infectious illness	5 (21%)	25 (35%)	0.30 (0.07-1.40)	0.126
	Respiratory tract	15 (63%)	43 (60%)	1.06 (0.31-3.60)	0.921
	Gastrointestinal tract	6 (25%)	10 (14%)	2.64 (0.70-9.95)	0.153
	Other	2 (8%)	3 (4%)	2.65 (0.33-21.11)	0.358

†high fever indicates a measured temperature ≥38°C (101°F)

Table 3. Number of infections and fever reports in all time points investigated

		Case Mean (SD)	Control Mean (SD)	OR (95 % CI)	p-value
Autoantibody	Any infection	3.5 (3.9)	2.8 (3.6)	1.22 (0.93-1.60)	0.150
negative period	Fever	1.0 (1.2)	1.1 (1.5)	0.97 (0.62-1.53)	0.901
Seroconversion	Any infection	1.7 (2.0)	2.0 (1.9)	0.93 (0.69-1.25)	0.615
period	Fever	0.3 (0.8)	0.8 (1.3)	0.61 (0.33-1.12)	0.108
Progression	Any infection	1.6 (1.5)	1.7 (2.1)	0.95 (0.66-1.37)	0.782
period	Fever	0.4 (0.9)	0.7 (0.9)	0.57 (0.26-1.21)	0.143

Figure Legends

Figure 1: Flow chart of the study population for the TEDDY book and sequencing study (A), Flow chart of the different time points investigated in the TEDDY book and sequencing study (B).