Online Appendix

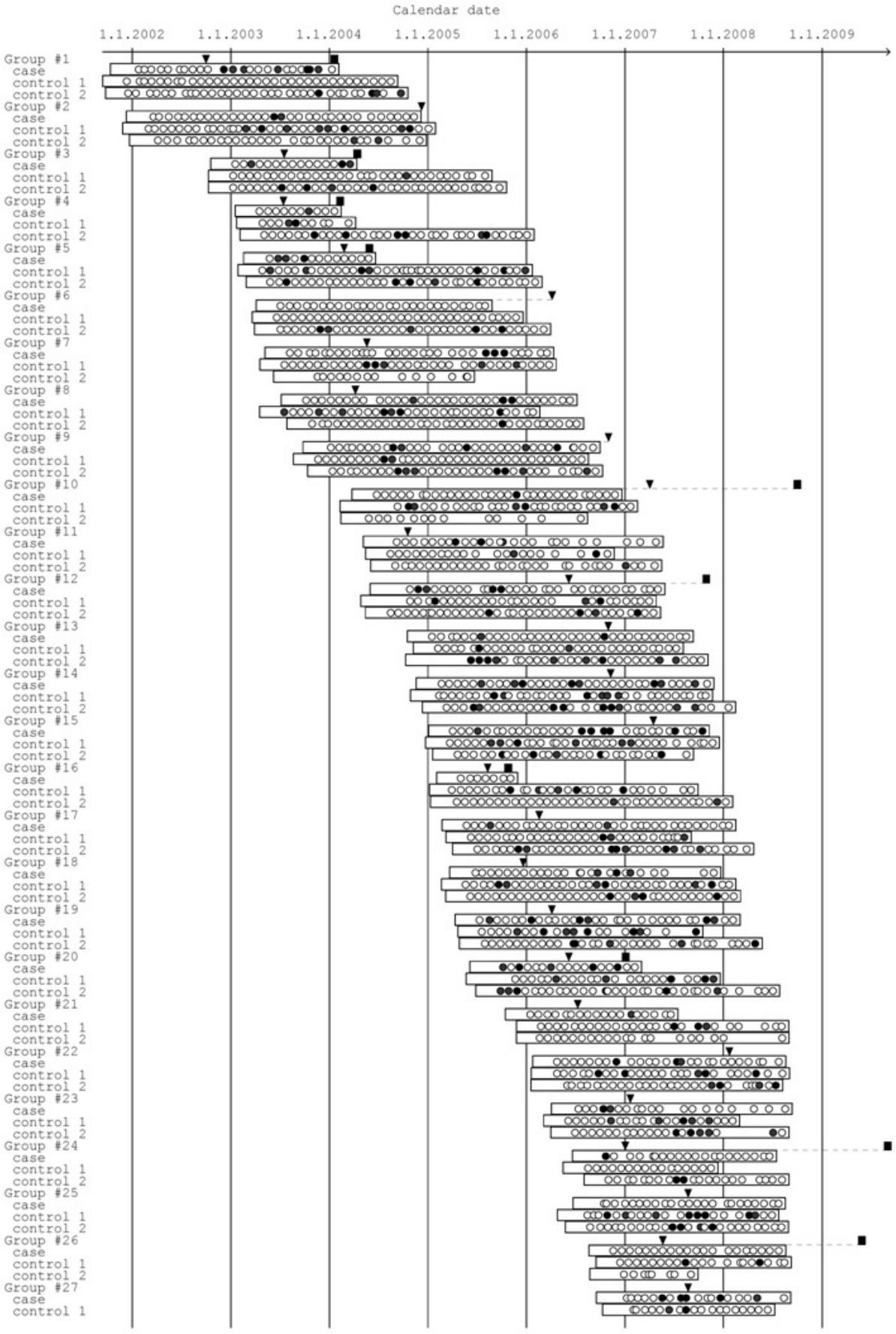
Supplementary Table 1. Age when first testing positive for specific islet autoantibodies and at diagnosis of type 1 diabetes among the 27 case children with islet autoimmunity. Case 17 might have reverted to a non-autoimmune state, as the last measurement was negative for all autoantibodies. Case 11 has had fluctuating levels of autoantibodies and was thought to have reverted to non-autoimmunity, but is again positive in the last measurement.

Matching Group Case	Anti-IAA (months of age)	Anti-GAD65 (months of age)	Anti-IA2A (months of age)	T1D (months of age)
1	12	12	15	27
2	51	36	42	-
3	-	12	15	18
4	6	9	9	13
5	15	12	-	15
6	36	-	42	-
7	15	12	48	-
8	18	9	24	-
9	42	36	48	-
10	36	36	36	54
11	18	12	-	-
12	24	24	-	41
13	-	24	27	-
14	24	30	-	-
15	24	30	30	-
16	6	6	9	9
17	12	30	33	-
18	9	9	12	-
19	18	12	18	-
20	12	12	15	19
21	9	9	15	-
22	-	24	24	-
23	12	9	27	-
24	18	12	-	-
25	12	6	12	31
26	9	9	18	33
27	9	12	12	-

Supplementary Table 2. Genotypes of human enterovirus detected.
The number of positive samples for each genotype is reported. As only a subset of samples was sequenced, direct comparison of genotype frequencies between cases and controls have limited relevance. The types are listed by their abbreviated name and number; CAV = Coxsackie A virus, CBV = Coxsackie B virus, E = Echovirus, ENV = Enterovirus

Genotype	Cases	Controls
CAV16	0	5
CAV2	0	3
CAV4	0	4
CAV5	2	2
CAV6	2	3
CAV9	4	8
CBV3	1	4
CBV4	5	7
CBV5	2	4
E11	2	2
E13	1	1
E18	1	5
E25	5	0
E3	4	10
E7	0	1
E9	1	4
ENV71	1	3
Total	**31**	**66**

Supplementary Figure 1. The subjects in the nested case-control study and their stool samples.



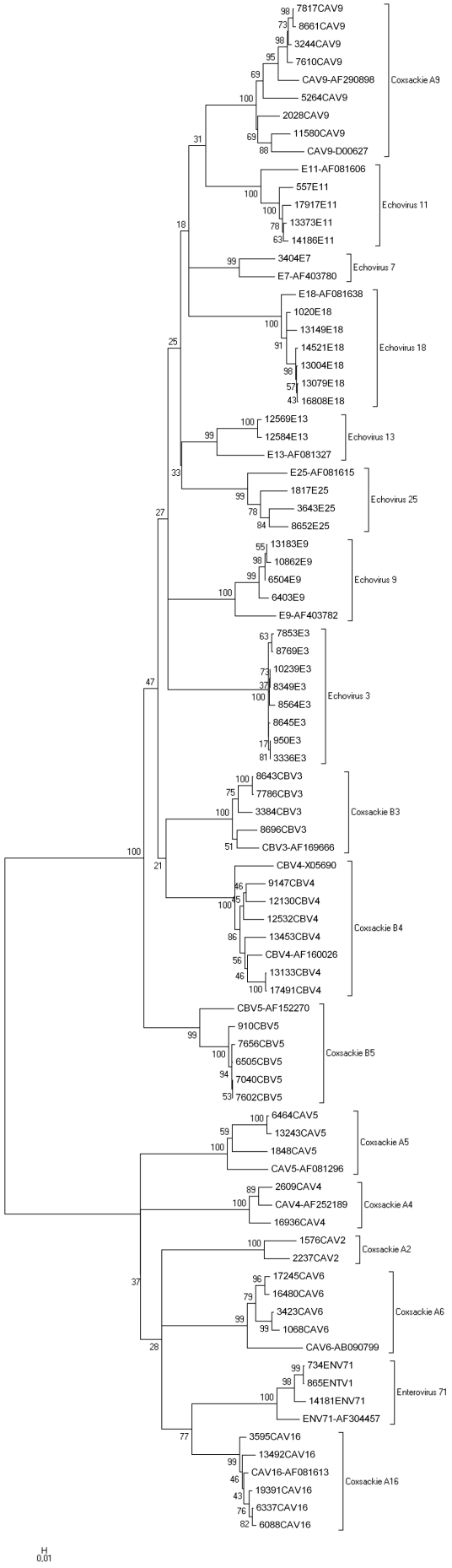
Twenty seven nested case-control trios are shown. The trios are ordered by the date of birth of the index case. The follow-up by stool samples for each child is indicated by the box framing the series of the circles depicting the stool samples obtained from each subject. The left edge of the box corresponds to the birth of the subject, the right edge to the last stool sample available (either from the 35th month of life when collecting of stool samples is terminated, or 17.8.2008, the date of the last stool sample processed for this study). The empty circles correspond to negative stool samples, grey to low -to-moderate enterovirus RNA quantity, and black to high-quantity positivity. Triangles show the dates of diagnosis of islet autoimmunity, i.e. the first of samples with one or more autoantibodies (of GAD, IA2, IAA) that was later confirmed by repeated positivity for two or more autoantibodies. Full squares show the date of diabetes onset. Diabetes was observed in 10 children by 1. September 2009. Note that control 2 of the matching group 27 withdrew in the course of study, and the data were therefore deleted.


Time point of islet autoimmunity development


Time point of type 1 diabetes diagnosed


Three stool samples:
First - negative (no enterovirus DNA detected or its quantity below 100 copies per microlitre RNA),
Second - positive at a low to moderate quantity (100 - 9 999 copies per microlitre RNA),
Third - positive at a high quantity (10 000 or more copies per microlitre RNA)

Supplementary Figure 2. Phylogenetic relationships in the VP1 regions.



Genotyping of enterovirus-positive samples. To distinguish between continuous infection and two distinct strains infecting the child in two consecutive months, enterovirus genotypes were determined in selected samples by partial sequencing of the VP1 region. First, a separate reverse transcription step was performed with a specific primer, using the Improm II chemistry (Promega, USA), followed by a nested PCR with primers published by Casas *et al.* (1). Direct product sequencing was performed using the inner amplification primer pair. Detailed protocols are available from the authors. The partial VP1 sequences were compared to published sequences in the GenBank database to determine whether the strain was genetically related to any known enterovirus type. The sequences were aligned and phylogenetic analysis performed, using the Molecular Evolutionary Genetics Analysis software, version 4.0 (2). In the multiple alignment analysis the Kimura two-parameter model was used as a model of nucleotide substitution, and the neighbor-joining method was used to reconstruct the phylogenetic tree, shown in Online Supplementary Figure 2. The statistical significance of the phylogenies constructed was estimated by bootstrap analysis with 1,000 pseudo replicate data sets, taken to represent the evolutionary history of the viruses. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Sequences from this study are marked with numeric sample code followed by the assigned type. Consecutive samples of the same genotype (from the same infection episode) are represented only once. Sequences from *Genbank* are marked with their type followed by the accession number. CAV: Coxsackie A virus, CBV: Coxsackie B virus, E: Echovirus, ENV: Enterovirus.

1. Casas I, Palacios GF, Trallero G, Cisterna D, Freire MC, Tenorio A: Molecular characterization of human enteroviruses in clinical samples: comparison between VP2, VP1, and RNA polymerase regions using RT nested PCR assays and direct sequencing of products. *J Med Virol* 65:138-148, 2001

2. Tamura K, Dudley J, Nei M, Kumar S: MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* 24:1596-1599, 2007