No Ljungan Virus RNA in Stool Samples From the Norwegian Environmental Triggers of Type 1 Diabetes (MIDIA) Cohort Study

OBJECTIVE — Ljungan virus (LjV) has been proposed as a potential environmental factor for type 1 diabetes. The objective was to test for any association of LjV with type 1 diabetes.

RESEARCH DESIGN AND METHODS — A nested case-control design was used to test for any association between the development of pre-diabetic autoimmunity and presence of LjV in stool samples (n = 3,803) in the Norwegian Environmental Triggers of Type 1 Diabetes (MIDIA) study. The children followed were 27 infants who developed pre-diabetic autoimmunity during or shortly after the sampling period, 54 matched control subjects, and 94 other children.

RESULTS — No LjV RNA was detected.

CONCLUSIONS — The results indicate that LjV is rare in young children. LjV does not seem to be involved in the development of human type 1 diabetes.

Diabetes Care 33:1069–1071, 2010

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CONCLUSIONS — The results indicate that LjV is rare in young children. LjV does not seem to be involved in the development of human type 1 diabetes.
The virus might reach the pancreas even through the intestinal tract and may cause autoimmunity observed in the present study. It seems unlikely that the virus is the cause of the autoimmunity observed in the earlier studies (5,6). In these studies, the presence of virus has been suggested partly by serology and partly by PCR; but, as pointed out by Bergström et al. (13), different methods do not seem to give congruent results. Moreover, the PCR positivity observed in the earlier studies is not reported to have been confirmed by sequencing. Although the evidence suggests possible human LjV infections, the data also indicate that it is a rare event and primarily during the prenatal period.

The likelihood of infection may also be geographically specific and dependent on the cycles of its natural reservoir, which presumably are bank voles in Scandinavia. Although common in Norway, their prevalence in the communities from which infants in the present study was recruited is not known. Thus, the possibility that the infants investigated were never exposed cannot be ruled out.

In conclusion, although the present data do not rule out the possibility that LjV can cause type 1 diabetes, they do suggest that this virus is not a common risk factor in the etiology of the disease in Norway.

Acknowledgments—This study and the MIDIA project were funded by the Norwegian Organization for Health and Rehabilitation (Grant 2005/2/0128), European Economic Area and Norway Grants (Grant A/CZ0046/11/0014 through the Research Support Fund, Prague, the Czech Republic), the Ministry of Education of the Czech Republic (Grant MSM0021620841), the Research Council of Norway (Grants 135893/330, 155300/320, 156477/730, and 166515/V50), the Norwegian Diabetes Association, the Children With Diabetes Foundation (Denver, CO), and New-Generics (Grant Food-CT- 2005-016320).

No potential conflicts of interest relevant to this article were reported.

We thank the public health care nurses for their effort in the recruitment to the MIDIA study and for the follow-up of high-risk children and the staff at the Biobank, Norwegian Institute of Public Health, for DNA extraction, and genotyping. In particular, we would like to express our gratitude to all the parents for their efforts in handling their child’s type 1 diabetes risk and for providing delivered blood and fecal samples from their children and completing questionnaires.

CONCLUSIONS—Considering the follow-up time, the number of tested samples, and that both pre-diabetic and healthy children were tested, these results indicate that LjV is very rare in the stool of Norwegian infants. The typical stool quantities of human enterovirus and HPeV in samples from the Norwegian Environmental Triggers of Type 1 Diabetes (MIDIA) cohort study were two to five orders of magnitude higher than the detection limit for LjV (9,12); presumably any appreciable replication of LjV in the gut would be detected. The detection of the exogenously added West Nile virus RNA safeguards against the presence of inhibitors and RNA degradation. The primers used consistently detected the positive LjV RNA controls included in each run and are expected to detect all strains of LjV. No change in sensitivity was detected with the introduction of a 96-well extraction method. The use of the antisense primer in the RT reaction could be presumed to increase the sensitivity at the cost of the formation of more spurious products, but was chosen to ensure that any possible positive sample would be detected. The lack of evidence for the presence of LjV suggests that this virus rarely infects the gut of Norwegian infants, and it seems unlikely that the virus is the cause of the autoimmunity observed in the present study.

Picornaviruses may also replicate outside the intestinal tract and may cause viremia or respiratory infections. Although one would expect a gastrointestinal route of infection in the case of LjV, the virus might reach the pancreas even after limited replication in the gut. Although data supporting LjV infections in humans have been published, there is so far no conclusive evidence. The arguably strongest evidence stems from prenatal studies (5,6). In these studies, the presence of virus has been suggested partly by serology and partly by PCR; but, as pointed out by Bergström et al. (13), different methods do not seem to give congruent results. Moreover, the PCR positivity observed in the earlier studies is not reported to have been confirmed by sequencing. Although the evidence suggests possible human LjV infections, the data also indicate that it is a rare event and primarily during the prenatal period.

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Figure 1—Age distribution of the samples. The number of samples from case children (■) and the total number of samples (□) distributed by month of age are shown.

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