The association between prior infection with five serotypes of Coxsackievirus B and incident type 2 diabetes mellitus in the EPIC-Norfolk study

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Abstract

Aims/hypothesis Infections with Coxsackieviruses have been linked to beta cell dysfunction. Given the importance of beta cell dysfunction in the aetiology of type 2 diabetes, we hypothesised that prior infection with Coxsackieviruses B would increase the risk of type 2 diabetes. The aims of the study were to estimate cross-sectional associations between potential predictors of previous infection and seropositivity for Coxsackievirus B serotypes 1–5 (CBV1–5), and then to assess the association between seropositivity and incident type 2 diabetes.

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Methods Using a case-cohort design nested within the European Prospective Investigation of Cancer (EPIC)-Norfolk study, we ascertained $n=603$ cases of incident type 2 diabetes. From within the entire cohort we identified a random subcohort of $n=835$, without diabetes at baseline. The presence of Coxsackievirus B neutralising antibodies against serotypes 1–5 was assessed using a plaque neutralisation assay. Weighted Cox regression was used to examine the association between presence of antibodies to CBV1–5 and the development of type 2 diabetes.

Results Seropositivity in the subcohort for CBV1–5 was 50%, 67%, 66%, 75% and 45%, respectively. After adjustment for age, sex, BMI, physical activity and family history of diabetes, the presence of antibodies against CBV1–5 was not associated with incident type 2 diabetes, over a mean follow-up of 5.7 years (HR [95% CIs] 0.94 [0.72, 1.25], 0.92 [0.68, 1.23], 1.33 [0.98, 1.81], 1.16 [0.83, 1.61] and 1.03 [0.77, 1.39] for CBV1–5, respectively).

Conclusions/interpretation The presence of antibodies against any of five serotypes of Coxsackievirus B was not associated with incident type 2 diabetes.

Keywords Cohort study · Coxsackievirus B · EPIC-Norfolk · Type 1 diabetes mellitus · Type 2 diabetes mellitus

Abbreviations

CBV1 Coxsackievirus B serotype 1
CBV2 Coxsackievirus B serotype 2
CBV3 Coxsackievirus B serotype 3
CBV4 Coxsackievirus B serotype 4
CBV5 Coxsackievirus B serotype 5
EPIC European Prospective Investigation
of Cancer-Norfolk

Introduction

Type 2 diabetes mellitus is increasing in prevalence [1], driven both by insulin resistance and beta cell dysfunction [2]. The importance of environmental risk factors in the aetiology of type 2 diabetes is well established [1], including individual behavioural factors such as physical activity and diet, and shared factors such as family history and socioeconomic status. Although many of these risk factors are inter-related, neither the association of family history nor that of socioeconomic status with type 2 diabetes are fully explained by health behaviours or by genetics, raising the possibility that other shared environmental risk factors may be important in driving the diabetes epidemic. One possible explanation for the marked socioeconomic gradient in type 2 diabetes risk is an infectious aetiology as many infections are socially patterned. Among the wide range of possible infectious causes, Coxsackieviruses are one of the most biologically plausible as they are known to be associated with beta cell dysfunction [3]. There is evidence suggesting enterovirus-driven pancreatic inflammation in type 2 diabetes as in a recent study 40% of samples of pancreatic tissue from type 2 diabetes patients (vs 13% of controls) stained with the enteroviral capsid antigen vp1 [4], and the levels of vp1 staining were associated with macrophage recruitment in the islets [5].

Despite their significance for human health, very few Coxsackievirus seroepidemiological studies have been undertaken. From the monitoring of sewage in Finland, there is evidence that the community circulation of enteroviruses is continuous and the most commonly detected in faeces are Coxsackieviruses B, serotypes 1–5 (CBV1–5) [6].

We aimed to estimate cross-sectional associations between various potential predictors of previous infection and seropositivity for CBV1–5 infections, and then to assess the association between seropositivity and incident type 2 diabetes.

Methods

Cohort characteristics We analysed data from a type 2 diabetes case-cohort study nested within the European Prospective Investigation of Cancer-Norfolk (EPIC-Norfolk) study. EPIC-Norfolk is a population-based cohort study that included 25,639 men and women aged 40–79 years who attended a baseline health check between 1993 and 1997. All volunteers gave written informed consent and the study was approved by the Norfolk Local Research Ethics Committee. Reported investigations have been carried out in accordance with the principles of the Helsinki Declaration.

Measurements, biochemical and haematological analyses A health and lifestyle questionnaire was completed at baseline, including questions on family history of diabetes, prescribed medications, occupational social class, smoking history, educational level and physical activity. A four-point physical activity index was developed and anthropometric measurements were taken according to standard protocol.

Blood samples, drawn in the non-fasted state, were analysed using standard assays. Researchers did not have access to identifiable information, and could only identify samples by number.

Type 2 diabetes case-cohort study characteristics Ascertainment of incident diabetes used six different sources of evidence, including linkage to primary care and hospital registers, hospital admissions and mortality data. Possible cases based solely on self-report did not qualify. Follow-up was censored at the earlier of the date of diagnosis, 31 July 2006, or the date of death. If the diagnosis date could not be ascertained, the midpoint between recruitment and censoring was used. Eight-hundred and ninety-two verified incident diabetes cases were identified. Individuals without stored blood were excluded, leaving a total of 603 incident cases. Cases of prevalent diabetes at baseline, identified on the basis of self-report of a history of doctor-diagnosed diabetes, diabetes drug use or evidence of diabetes after baseline with a date of diagnosis earlier than the recruitment date, were excluded from the analysis.

A random subcohort of 872 individuals was selected from within the entire cohort. After the exclusion of 27 prevalent cases of diabetes, a subcohort of 835 individuals remained. This subcohort included (by design) 40 individuals who had developed incident diabetes during follow-up.

Mean time of follow-up was 5.8 years (SD 5.3).

Detection of neutralising antibodies To identify antibodies for each of the five Coxsackievirus serotypes, plaque neutralisation was performed by the University of Tampere, using virus strains obtained from the American Type Culture collection. Serum was administered onto green monkey kidney cell monolayers. Presence of neutralising antibodies ('seropositivity') against the corresponding serotype was identified if plaque formation was decreased by 80%; the opposite was considered seronegativity. The assay used has a within-run repeatability of 100% and in-house reproducibility using different virus stock of 99% and has also been previously validated in patients with acute enterovirus infection diagnosed by alternative methods.

Statistical analysis We summarised baseline characteristics of the subcohort and all the incident type 2 diabetes cases

using means and SD for continuous variables, and percentages for categorical variables. We used a χ^2 test to investigate the relationship between age (grouped in decades) at the time of entry to the study and presence of antibody for CBV1–5. We assessed agreement between seropositivity for Cocksackievirus serotypes using the κ statistic.

We used logistic regression to estimate cross-sectional associations between various baseline characteristics and seropositivity within the subcohort, adjusted for age and sex. We used Prentice-weighted Cox regression to estimate HR for incident type 2 diabetes, comparing seropositivity with seronegativity for each serotype as well as seropositivity for more than four compared with less than four serotypes, adjusting for age (as the underlying timescale), sex, BMI, physical activity and family history of diabetes.

Statistical analyses were performed using Stata/SE 11.2 (Stata-Corp, College Station, TX, USA).

Results

Characteristics of the subcohort and all the incident type 2 diabetes cases are presented in electronic supplementary material (ESM) Table 1.

Seropositivity in the subcohort for CBV1–5 was 50%, 67%, 66%, 75% and 45%, respectively. Agreement between positivity for different serotypes was weak (κ 0.091). Seropositivity for Cocksackieviruses B by decade of age at baseline is presented in ESM Fig. 1. Of the baseline characteristics examined, only increased LDL-, decreased HDL- and increased total to HDL-cholesterol ratio were significantly associated with seropositivity for one or more of the Cocksackievirus B serotypes (ESM Fig. 2).

There was no evidence of association between seropositivity and incident type 2 diabetes (Table 1). There was no effect of having antibodies for four or more serotypes versus less than four (HR [95% CI] 1.23 [0.92, 1.64]).

Discussion

This is the largest study examining the seroepidemiology of Cocksackieviruses B in the UK. A large percentage of adults lack antibodies against multiple Cocksackievirus serotypes and lack, therefore, protection against future clinical disease.

Our study did not show an association between previous Cocksackievirus B infection and new-onset type 2 diabetes. The assay performance suggests that our negative finding is not due to laboratory imprecision. In addition, our study was sufficiently powered to detect modest levels of association but it is always possible that we have missed a weak association, but this would be unlikely to be clinically relevant.

Any diabetogenic effects of Cocksackieviruses B could be different by age. Early cases of type 2 diabetes with an autoimmune component could have been excluded due to the cohort entry age restrictions and this might have diluted an overall effect. A loss of seropositivity in older participants is less likely to have affected our results as there is no convincing pattern of lower seropositivity in older groups.

Our assay cannot discriminate between multiple infections by the same serotype. It is possible that both a particular genetic make-up of the host and the appropriate strain are necessary for diabetogenesis, as proposed by Roivainen et al [7]. In addition, studies in the past have revealed that, at least for some individuals, infection with a different Cocksackievirus B serotype can increase the levels of antibody against a previously infecting serotype (heterotypic response) [8]. The lack of uniformity of presence of antibodies against all serotypes at old ages argues against heterotypic antibody responses being common.

The consistency of the association with lipids is intriguing. Lipoproteins have antiviral properties [9]. These observed cross-sectional associations are likely to be due to chance but could also be explained by decreased susceptibility to infection among individuals with constitutively higher lipid subfractions or a virus dyslipidaemic effect. The above observation could be important when attempting to interpret the association of infection with cardiovascular

Table 1 HR and 95% CI for the association between different Cocksackievirus B serotypes and incident type 2 diabetes in the EPIC-Norfolk type 2 diabetes case-cohort study ($N=1,398$)

Model adjustments	Age ^a	Age ^a , sex	Age ^a , sex, BMI	Age ^a , sex, BMI, physical activity ^b	Age ^a , sex, BMI, physical activity ^b , family history of diabetes
CBV1	1.01 (0.81, 1.26)	1.03 (0.82, 1.29)	0.95 (0.73, 1.25)	0.92 (0.69, 1.22)	0.94 (0.71, 1.25)
CBV2	1.11 (0.88, 1.39)	1.14 (0.89, 1.45)	0.92 (0.70, 1.19)	0.90 (0.69, 1.18)	0.92 (0.68, 1.23)
CBV3	1.12 (0.89, 1.42)	1.14 (0.89, 1.44)	1.35 (1.00, 1.81)	1.34 (0.99, 1.80)	1.33 (0.98, 1.81)
CBV4	1.14 (0.88, 1.46)	1.13 (0.88, 1.45)	1.09 (0.80, 1.50)	1.15 (0.84, 1.59)	1.16 (0.83, 1.61)
CBV5	0.98 (0.77, 1.22)	0.96 (0.76, 1.20)	0.99 (0.74, 1.33)	1.01 (0.74, 0.97)	1.03 (0.77, 1.39)

^a Age is the underlying timescale in the Cox model.

^b Physical activity is assessed by a four-point activity index, ranging from 1 for the most sedentary to 4 for the most active.

disease, as the levels of cholesterol subfractions might be modified by viruses.

Conclusion

Seropositivity for CBV1-5 in the EPIC-Norfolk cohort ranged from 45% to 75% and was not associated with incident type 2 diabetes.

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Duality of interest H. Hyöty is a shareholder and member of the Board of Vactech Ltd, which develops vaccines against picornaviruses.

Contribution statement All authors contributed to the study conception and design, analysis and interpretation of data and approved the final version of the article to be published.

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