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Maternal exposure to air pollution and type 1 diabetes - accounting for genetic factors

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Abstract

Background: Genetic and non-genetic factors probably act together to initiate and accelerate development of type 1 diabetes [T1D]. One suggested risk factor contributing to development of T1D is air pollution.

Objective: The aim of the study was to investigate whether maternal exposure during pregnancy to air pollution, measured as nitrogen oxides [NO_x] and ozone, in a low-dose exposure area was associated with the child developing T1D.

Method: In Scania (Skåne), the most southern county in Sweden, 84 039 infants were born during the period 1999-2005. By the end of April 2013, 324 of those children had been diagnosed with T1D. For each of those T1D children three control children were randomly selected and matched for HLA genotype and birth year. Individually modelled exposure data at residence during pregnancy were assessed for nitrogen oxides [NO_x], traffic density and ozone.

Results: Ozone as well as NO_x exposures were associated with T1D. When the highest exposure group was compared to the lowest group an odds ratios of 1.62 (95% confidence interval [CI] 0.99 to 2.65) was observed for ozone in the second trimester and 1.58 (95% CI 1.06-2.35) for NO_x in the third trimester.

Conclusion: This study indicates that living in an area with elevated levels of air pollution during pregnancy may be a risk factor for offspring T1D.

Key words: Diabetes Mellitus Type 1, Type 1 Diabetes Mellitus, Air Pollution, Nitrogen Oxides, Ozone, Maternal Exposure

Introduction

Type 1 diabetes [T1D] is the result of autoimmune destruction of insulin-producing pancreatic islet beta cells. It is a serious condition where survival depends on daily insulin injections. The incidence of T1D in Sweden is the second highest in the world, surpassed only by Finland (Green *at al.* 2001, Karvonen *et al.* 2000). The aetiology of T1D is only partly understood, but it is widely accepted that genetic and non-genetic factors act together to initiate and accelerate the development of T1D (Daneman 2006; Lernmark 1999). One hypothesis is that T1D results from the action of environmental factors on genetically predisposed individuals (Mehers and Gillespie 2008).

There is more evidence for the genetic risk of the disease than the potentially contributing environmental factors (Regnéll and Lernmark 2013). The Human Leukocyte Antigen [HLA] system is linked to the immune system and is located on chromosome 6p21. It is a highly variable region of the human genome, and HLA haplotypes can act in both a risk-enhancing and protective way. One or both of the susceptibility haplotypes with alleles at the DQA1 and DQB1 loci are present in 90-95 % of young children developing T1D, whereas the protective haplotype is present in less than 0.1% of T1D cases (Regnéll and Lernmark 2013; Mehers and Gillespie 2008) . The main mechanism of action of these molecules is their peptide-binding activity in antigen-presenting cells (Regnéll and Lernmark 2013).

But only 6% of the individuals with the highest known genetic risk of developing T1D actually develop T1D, emphasising the importance of environmental triggers in disease development (Ilonen et al. 2002). Several perinatal environmental factors have been suggested to trigger the development of T1D, such as viral infections, intrauterine growth and diet, although none of them has been unequivocally confirmed (Regnéll and Lernmark 2013).

Studies from the US have given the first indications that air pollution may be a risk factor for T1D (Hathout *et al.* 2002; Hathout *et al.* 2006). Hathout and colleagues found an association between early-life exposure to sulphate (SO_4^{2-}) and ozone (O_3), with increased risks of developing T1D (odds ratios [OR] of 2.89 and 1.65, respectively). Exposure to particles, measured as PM_{10}, was associated with the development of T1D before the age of 5 years with an OR of 3.32 (Hathout *et al.* 2002). To date, no further studies in this field, to the best of the authors' knowledge, have been performed

Evidence is scarce regarding sensitive exposure windows of the hypothesised association between environmental factors and the risks of developing T1D. It has been suggested that T1D is likely to originate from gene-environment interactions during foetal development (Howard *et al.* 2011). Recent evidence provides a convincing link between a suboptimal gestational environment and an increased risk of onset of metabolic diseases (Joss-Moore and Lane 2009). Most cases (94%) of T1D can be predicted by detecting multiple islet autoantibodies in children below the age of five years, supporting the hypothesis that the autoimmune processes occur early in life (Regnéll and Lernmark 2013; Mehers and Gillespie 2008).

The aim of the present case-control study was to investigate the association between maternal exposure to air pollution during different periods of pregnancy and T1D when genetic predisposition was taken into account.

Method

Study area

Scania (Skåne) is the southernmost county in Sweden (Figure 1), covering an area of around 11350 km^2 (about 2% of the total area of Sweden). It is one of the most densely populated

areas of the country, with about 1.1 million people (approximately 11% of the total Swedish population). The level of air pollutants in the western part of Scania (where most people live) is higher than in other areas of Sweden, due to road transport to and from the European continent and a considerable amount of cargo shipping and ferry transport along the coast. However, annual mean NO_x on the west coast of Scania in 1999 was around 20 $\mu g/m^3$ (Sjöberg *et al.*2006), which is well below the present-day WHO air quality guideline value of 40 $\mu g/m^3$ (WHO 2005), and levels of ozone are around 50 $\mu g/m^3$, well below (health) guideline value of 120 $\mu g/m^3$.

Study population

In Scania all children born between 1999 and 2005 diagnosed with T1D have been registered, in the Skåne study (1999-2005), and the Better Diabetes Diagnosis study (2005 onward) regardless of whether the child has moved to another region since birth or not. T1D diagnosis is established according to guidelines from the American Diabetes Association. Out of a total 84 039 children, 341 children were diagnosed with T1D prior to 1 May 2013.

From September 2000 to August 2004 all children born in Scania were offered testing for genetic risk of T1D at birth in the DiPiS project [Diabetes Prediction in Scania] with the aim of determining genetic as well as environmental risk factors before and after pregnancy that may trigger T1D. A total of 35 658 out of 48 058 children born during that period were HLA-typed in the DiPiS project Step 1. HLA-genotyping was performed on dried blood spots described in more detail in (Larsson et al. 2004). Filter samples (3 mm in diameter) of DBS were punched into 96-well PCR plates and transferred to DELFIA streptavidin-coated microtitration plates (Perkin Elmer Life Sciences, Boston, MA, USA) and diluted with DELFIA hybridisation buffer. After incubation and denaturation the single-stranded DNA was hybridised with the following probes (Perkin Elmer Life Sciences): the first set contains

Eu-DQB1*0602/3, Sm-DQB1*0603/4 and the second one contains Eu-DQB1*0302, Sm-DQB1*0301 and Tb-DQB1*02. The samples positive for DQB1*02 were further analysed for DQA1*0201 and 05 alleles to separate DR3 from subjects with DR7. HLA-DQA1 typing used Sm-DQA1*05 and Tb-DQA1*0201 probes. After washing and addition of DELFIA enhancement solution, the Eu and Sm fluorescence was counted in a Victor2 MultiLabel Counter (Perkin Elmer Life Sciences). The Tb signal-to-noise ratio was calculated using MultiCalc (Perkin Elmer Life Sciences).

Children with a high risk of disease were followed in Step 2 (see Figure 1). From the DiPiS Step 2 cohort three children without T1D were randomly selected for each case, matched for HLA genotype and birth year. We performed as exact HLA matching as possible. A match was considered exact if the case child and the control had two of the same DQA1 alleles and the same DQB1 alleles. Three exact matches was available for 130 (38%) of the cases, 148 (43%) had two exact matches, 53 (15%) had one exact match and for 13 (4%) of the cases no exact match was available. Where no exact match was possible a control with random HLA was chosen. For children with T1D born in 2000-2004 and participating in DiPiS, HLA-genotyping was done at birth, and for non-participating children born 1999-2005, HLA-genotyping was done at the time of diagnosis. A more detailed description of the study population is illustrated in Figure 1.

Six hundred and eighty-two controls were exactly matched for HLA, while the other 262 controls were matched due to HLAs with similar risks. We further matched the controls for birth date of the child, and 62% of the controls were born ± 1 year in comparison to their cases and 94% within two years. By linking to the Swedish Medical Birth Registry (SMBR) we obtained information about potential confounders.

This study was approved by the Lund University Ethics Committee.

Outcome measures

In the study area all children with T1D are diagnosed according to the standards set by the American Diabetes Association (American Diabetes Association 2011). Since presence of islet auto-antibodies precedes manifest clinical onset of T1D, we only included control children who had not tested positive for auto-antibodies (more than one) against the 65 kD isoform of glutamic acid decarboxylase (GADA), the protein tyrosine phosphatase-related IA-2 antigen (IA-2A), insulin (IAA) and the zinc transporter 8 (ZnT8A) which could indicate early signs of disease (Regnéll and Lernmark 2013). We used only controls who had been tested no later than 1.5 years prior to 1 May 2013.

Exposure assessments

Modelled NO_x exposure

For NO_x exposure we used an Emission Data Base (EDB) combined with a sophisticated Gaussian dispersion model with a resolution of 500*500m. The EDB for NO_x in Scania is extensive due to the legislation requiring polluters to report emissions. It contains information on emissions from approximately 24 000 sources (Stroh *et al.* 2005). Most of these sources are line sources corresponding to road traffic, shipping and railways. Point sources include industrial installations and larger energy and heat producers. Area sources included are emissions for example from aviation, small-scale heating and construction machinery. Emissions from eastern Denmark have also been added into the EDB, since emissions from eastern Denmark are quite high and westerly winds are dominant (Kristensson *et al.* 2008). The EDB was combined with a modified Gaussian dispersion model, AERMOD (U.S. Environmental Protection Agency 2004), used for dispersion calculations. AERMOD is a flat two-dimensional model taking the height of the emission source as well as meteorology into

account. To account for long-range transboundary air pollution, we have added a background level of 2.5 μg/m^3 into the model (corresponding to the yearly mean from a local remote background monitor station). For validation, the modelled concentrations from our air pollution model have been plotted against measured façade concentration. A diffusion sampler was attached to 86 residences for a week and 64 residents agreed to participate for an additional week, providing a total of 150 measurements at residences. The association between modelled and measured levels at residences was strong, with a Spearman correlation of 0.8 (Stroh *et al.* 2012).

In this present study the concentrations of NO_x were modelled as hourly means using a spatial resolution of 500x500m. These hourly means were aggregated to individually calculate gestational monthly and trimester means (months 1-3 of pregnancy, months 4-6 and month 7-delivery). In this EDB, the spatial resolution of 500x500m has been shown to be appropriate, with regard to accuracy, when studying aggregated monthly means (Stroh *et al.*2007).

We used a categorical classification of NO_x by dividing the exposure levels into categories based on overall data from the first trimester for all births in the area between 1999 and 2005 in accordance with previous studies (Malmqvist et al. 2011; 2013), giving the following cut-off values (in ppb using the WHO conversion rate for NO_2):

- 2.5-8.9 μg/m^3 – reference category (1.3-4.7 ppb)
- 9.0-14.1 μg/m^3 (4.8-7.5 ppb)
- 14.2-22.6 μg/m^3 (7.6-12 ppb)
- >22.7 μg/m^3 (>12.1 ppb)

Ozone

We used measurements of ozone from 30 stations (background and urban background), and all individuals were assessed with measurements using the nearest station approach. If the nearest station had missing data the individual was assessed at the second-nearest station or the third-nearest station. We excluded data if there was more than 10% missing data or if an individual lived further away from a station than 32 km according to the EPA recommendations (Abbey et al. 1991). The mean distance to stations used in the model was 8.5 km, with a range from 0.1 to 32 km. We used 24 hour average ozone data and aggregated into trimester data. Ozone in the study area has been extensively studied and in transport of ozone (and precursors) local production dominates, leading to small spatial variation (Malmqvist et al. 2014).

The ozone levels were categorised into quartiles based upon the first trimester distribution among the controls in the study (in ppb using the WHO conversion rate for Ozone):

- <43 μg/m^3 - reference category (<22 ppb)
- 43-52 μg/m^3 (22-26.5 ppb)
- 52.1-60 μg/m^3 (26.6-30.6 ppb)
- >60 μg/m^3 (>30.6 ppb)

Linking to the exposure databases

Each resident in Sweden has a unique 10-digit personal identification code, which can be linked to the spatial coordinates of their place of residence (yearly updated). Individuals are positioned according to the centre coordinate of their place of residence. We linked the mother's residential coordinates to the air pollution databases in order to individually assess exposure. With the maternal personal identification code we were able to link the mother's exposure to her information in the Swedish Medical Birth Registry [SMBR] and with her child in the diabetes registries.

Potential confounders

Factors which have been suggested as having an association with an altered risk of developing T1D during foetal development are preeclampsia (Jones *et al.* 1998), maternal age, birth weight and parity (Dahlquist *et al.* 1999), gestational length (Locatelli *et al.* 2007), maternal diabetes and maternal smoking (Marshall *et al.* 2004) and sex of the child (Svensson *et al.* 2005). It has also been suggested that ethnicity can be a risk factor, with a higher incidence in those of European descent (Borchers *et al.* 2010) which is even further increased in those of Swedish descent (Dahlquist *et al.* 1996; Hjern and Söderström 2008). With the exceptions of viral infections and diet, we obtained information for the other potential confounders from the Swedish Medical Birth Registry (SMBR) (Socialstyrelsen 2002).

Statistics

Conditional logistic regressions, taking the matched design into account, were used for evaluation of the associations between maternal exposure to air pollution and the odds of developing T1D. We used IBM SPSS version 21 (IBM 2013) and LogXact (Cytel Studios 2013). The results were considered as statistically significant if the 95 % confidence interval [CI] did not include 1.

The robustness of the results was evaluated by sensitivity analyses where we excluded children of mothers who i) had T1D, ii) had gestational diabetes, iii) had been born in another country, or iv) smoked and v) children born Large for Gestational Age [LGA]. As an additional sensitivity analysis we excluded cases and controls that were not exactly HLA matched. We also performed multivariate analyses including the following variables; maternal T1D, gestational diabetes, preeclampsia, maternal country of origin, smoking and LGA.

Furthermore, as case children come from both the DiPiS cohort and the regional registries, while the controls come only from the DiPiS cohort, we were concerned that there might be

some selection bias. We therefore investigated the air pollution exposure prevalence including only case children who had participated and been diagnosed with T1D within the DiPiS project, see Figure 1. We also aimed to repeat all the analyses above in this subsample, but due to the small sample size (case children, n=49) and the few children with T1D who were exposed to the highest quartile of exposure prenatally (n=8), models could not be fitted to the data.

Results

Background characteristics and exposure distributions among those children who had (n=324) and those who had not (controls, n=930) developed T1D are shown in Table 1. Children with T1D had more often been exposed prenatally to the highest NO_x quartile (18%) than those without T1D (14%). When restricting to children in the DiPiS dataset, 16% of the cases and 14% of the controls were exposed to the highest NO_x exposure category.

When we performed gene-matched case-control logistic regressions the effects were more pronounced. Regarding NO_x exposure the ORs were above one for all three trimesters (1.36, 1.38 and 1.58, respectively, Table 2) when we compared the highest exposure category with the reference category. However, it was only exposure during the third trimester that reached statistical significance (OR 1.58 95% CI 1.06 to 2.35). When only the exact HLA-matched case-control sets were included in the analyses the corresponding OR was 1.47 (95% CI 0.96 to 2.24). The estimates only changed marginally when we performed the sensitivity analyses (data in Table 3). In multivariate analyses the adjusted ORs for developing T1D when comparing highest with lowest category of exposure were 1.38 (95% CI 0.98 to1.93) for NO_x (data in Table 4).

For ozone exposure during the second trimester an association, albeit not statistically significant, with T1D was observed when the highest exposure quartile was compared with the reference category (OR 1.62, 95% CI 0.99 to 2.65; Table 2). When only the exact HLA-matched case-control sets were included in the analyses the corresponding OR was 1.71 (95% CI 1.00 to 2.93). No significant associations were observed for ozone exposures during trimester 1 and trimester 3, although the corresponding ORs were above one. Again, the estimates only changed marginally when we performed the sensitivity analyses (data in Table

3). In multivariate analyses the adjusted ORs for developing T1D when comparing highest with lowest quartile of exposure were 1.56 (95% CI 1.04 to 2.35) for ozone (data in Table 4).

Discussion

The main finding in our study is that the mothers of offspring who developed T1D more often had lived in areas of elevated levels of NO_x during the third trimester or ozone during the second trimester, compared to mothers of children who had not developed T1D.

The major strength of this study is that in a population-based prospective study we have been able, for the first time, to analyse the association of air pollution during pregnancy and childhood T1D after controlling for the genetic risk of disease. We were able to model exposure to air pollution with high spatial resolution, and had information on other suggested perinatal risk factors. During the study period T1D has been thoroughly investigated and registered, and accordingly all children with T1D should have been captured. The children in this cohort are between 8 and 14 years old and controls could hypothetically develop disease later on in life, leading to a possible misclassification bias. However, controls participated in prospective follow-up and did not have T1D-associated autoantibodies, thus minimising the risk of misclassification bias.

Ritz (2010) discussed possible mechanisms for linking air pollution to autoimmunity disorders, such as T1D. If air pollution can influence the immune system and trigger other hypersensitivity disorders, such as allergy, it is, according to Ritz (2010), an indication that more research in the field of autoimmunity and air pollution is needed. The possible biological mechanisms through which air pollution could influence the development of T1D could be through the role of oxidative stress, which could lead to the production of pro-

inflammatory cytokines and thus spill over to the systemic circulation and facilitate autoimmune responses (Ritz 2010).

As in most epidemiological air pollution studies exposure is only measured outdoors at the residential address, which might not reflect indoor pollution levels. An inherent problem is also that the NO_x exposures are strongly correlated with urban-rural contrasts, and there may be other unmeasured confounders that we cannot take into account. The main concern in performing an epidemiological study on the odds of developing T1D is that although many risk factors have been suggested none has been unequivocally confirmed, and it is thus hard to know which risk factors to account for. In this study we chose to perform separate analyses where we excluded participants with a potential risk factor that differed in incidence between cases and controls. One such risk factor was maternal smoking, which in our study was associated with T1D (Mattsson et al. 2015). Other potential risk factors such as milk or viral infections could not be accounted for but are not unequivocally confirmed, and the only likely link to exposure would be the seasonality of viral infections and ozone levels.

The previous studies performed (Hathout *et al.* 2002, Hathout *et al.* 2006) looked at higher levels of pollutants and during childhood rather than during pregnancy. Sulphate [SO_4^{2-}] and ozone (O_3), were associated with considerably increased risks of developing T1D (odds ratios of 2.89 and 1.65, respectively). There is no correlation between O_3 and SO_4^{2-} and NO_x, while a strong correlation is seen between NO_2 and NO_x. Hathout did not find any statistically significant effects of NO_2 on the risk of developing T1D. The present study should be viewed in the perspective that it was performed in a relatively low-dose area with air pollution levels generally below current air quality guidelines (Malmqvist et al. 2013). We did not study the effect of SO_4^{2-}, since levels of SO_4^{2-} were low (5 $\mu g/m^3$ as compared to AQG 20 $\mu g/m^3$) and

with small spatial variation (Sjöberg et al. 2006). Unfortunately, due to lack of data we could neither study the association of particles on the risk of developing T1D. However, we do believe that the association we see with NO_x could be attributed to NO_x as a marker of ultrafine particles.

It should be mentioned that the ozone exposure assessment is rather crude. However, in our area ozone has been studied extensively and ozone tends to vary more in time than in space (Malmqvist et al. 2014). Thus, the effect we see is probably a seasonal effect of ozone and we could therefor see an effect of both NO_x and ozone (which otherwise tend to anti-correlate). Furthermore, our limited exposure assessment method for ozone was only available for 203 out of the 324 cases which should be considered when interpreting results. We can only speculate on why we see an effect in the second trimester but not in the other trimesters, but it could be linked to the maturation of the immune system and the lymphatic system. Ozone has strong oxidative potential due to its chemical composition and could affect the immune or lymphatic system by oxidative stress.

Moreover, we suggest that the effect we see is due to prenatal exposure to air pollution. It can, however, be argued that if a large proportion of mothers have not moved since pregnancy the NO_x exposure distribution remains and we thus cannot differentiate exposure during pregnancy from exposure during the first years of life.

More studies in the field are needed in both low-dose and high-dose areas, during different time windows, taking different pollutants into the model and with careful consideration of exposure assessment before any definite conclusion can be drawn.

Conclusions

The main finding in our study is that the mothers of offspring who developed T1D more often had lived in areas of elevated levels of NO_x during the third trimester or ozone during the second trimester, compared to mothers of children who had not developed T1D. Future studies are needed to confirm the findings.

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