EXTENDED REPORT

Perinatal and early childhood risk factors associated with rheumatoid factor positivity in a healthy paediatric population

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Objective: To examine perinatal and childhood risk factors for the presence of rheumatoid factor in healthy children.

Methods: The Diabetes Autoimmunity Study in the Young (DAISY) is a longitudinal study of children at increased risk of type 1 diabetes, based on possession of human leucocyte antigen (HLA)-DR4 and DR3 alleles or a family history of diabetes. 651 children who participated in DAISY, with an average age of 6.4 (range 1–15) years, were tested for the presence of rheumatoid factor in their most recent serum sample. 23 children were positive for rheumatoid factor. Exposure data were collected prospectively by interview. HLA-DR4 alleles were identified using polymerase chain reaction-based Class II genotyping.

Results: While exploring risk factors for rheumatoid factor positivity in a multivariate model, several important interaction terms involving HLA-DR4 status suggested the need to evaluate risk factors in HLA-DR4-positive and HLA-DR4-negative children separately. In HLA-DR4-negative children, rheumatoid factor-positive infants were less likely to have been breast fed for >3 months (odds ratio (OR) 0.18; 95% confidence interval (CI) 0.04 to 0.99), more likely to have been exposed to non-parental tobacco smoke (OR 5.38; 95% CI 0.93 to 31.27) and more likely to be a race/ethnicity other than non-Hispanic white (OR 6.94; 95% CI 1.10 to 43.88) compared with rheumatoid factor-negative children, after adjusting for age, sex and maternal education. In HLA-DR4-positive children, there were no significantly associated risk factors for rheumatoid factor positivity.

Conclusions: Risk factors for rheumatoid factor positivity in children vary by HLA-DR4 genotype. In HLA-DR4-negative children, breast feeding may decrease the risk, and environmental tobacco smoke may increase the risk, of autoimmunity.

The presence of rheumatoid factor is a well-established test used in the diagnosis and prognosis of rheumatoid arthritis. In addition, rheumatoid factor precedes rheumatoid arthritis,^{1–3} and adults with stable increases in rheumatoid factor have an increased incidence of rheumatoid arthritis.⁴ Studies on healthy adults using rheumatoid factor as an intermediate phenotype have shown that rheumatoid factor positivity is associated with a lack of oral contraceptive use⁵ and smoking,^{5–7} suggesting that these factors may play an important early part in the pathogenesis of adult-onset rheumatoid arthritis. Rheumatoid factor is also associated with polyarticular juvenile rheumatoid arthritis (JRA),^{8,9} the subtype of JRA that is most similar to adult-onset rheumatoid arthritis in terms of disease manifestation. Examination of the risk factors for rheumatoid factor positivity in healthy children may yield important clues as to the early pathogenesis of polyarticular JRA or, potentially, seropositive rheumatoid arthritis in general.

Studies on the environmental risk factors for JRA have been few and inconsistent. One study found that children with JRA were less likely to have been breast fed than controls,¹⁰ whereas others found no association with breast feeding.^{11,12} One study has linked maternal smoking during pregnancy with increased risk of JRA,¹³ and another reported an increased rate of current parental smoking among a group of children diagnosed with JRA.¹⁴ Given that these retrospective studies are potentially subject to recall and selection biases, examination of risk factors using prospectively collected data in a population not selected on the basis of disease status is warranted to provide a clearer picture about perinatal and early childhood exposures. However, the low prevalence of JRA makes it prohibitive to conduct a prospective study of this disease, suggesting the need

to examine intermediate phenotypes such as the presence of rheumatoid factor.

In our current study, we examined perinatal and early childhood risk factors for the presence of rheumatoid factor in a cohort of healthy children at increased genetic risk of rheumatoid arthritis, polyarticular JRA and type 1 diabetes autoimmunity (T1DM) due to an increased prevalence of the human leucocyte antigen (HLA)-DR4 allele. In contrast with most of the previous studies, we used prospectively collected data to determine environmental exposure associations with autoimmunity.

METHODS

The Diabetes Autoimmunity Study in the Young (DAISY) is a prospective study of the natural history of environmental risk factors for T1DM in genetically predisposed children. Children born at St Joseph's Hospital, Denver, Colorado, USA, whose population is representative of the general population of the Denver area, were identified by screening umbilical cord blood samples for diabetes susceptibility alleles in the HLA region. Families were excluded if the parents had difficulty understanding English, or if the infant had a congenital malformation or disease. About 86% of families gave informed consent for genetic screening. Over 28 000 cord blood samples have been screened for HLA genotypes at Roche Molecular Systems, Alameda, California, USA. Details of the newborn screening have been published elsewhere.¹⁵ Children were asked to

Abbreviations: BMI, body mass index; DAISY, Diabetes Autoimmunity Study in the Young; HLA, human leucocyte antigen; JRA, juvenile rheumatoid arthritis; T1DM, type 1 diabetes autoimmunity

participate in DAISY if their HLA genotypes included: DRB1*04, DQB1*0302/DRB1*03, DQB1*0201; DRB1*04, DQB1*0302/DRB1*04, DQB1*0302; DRB1*04, DQB1*0302/x (where x is not DRB1*04, DQB1*0302, DRB1*03 or DR2); and DRB1*03, DQB1*0201/DRB1*03, DQB1*0201. These genotypes containing DRB1*04 are associated with increased risk of rheumatoid arthritis and polyarticular JRA.

In addition to the HLA-screened newborn population, children who had a sibling or parent with T1DM were identified from families attending clinics in the Denver metropolitan area (mostly from the Barbara Davis Center for Childhood Diabetes, University of Colorado at Denver and Health Sciences Center, Denver, Colorado, USA), and were recruited regardless of their HLA genotype. These children could be enrolled in the cohort at any age from birth to 8 years. Participants were scheduled for blood draws at enrollment, and annually thereafter. Informed consent was obtained from parents of all children in DAISY, approved by the Colorado Multiple Institutional Review Board.

Measurement of exposures

Sociodemographic factors and perinatal variables were determined by a questionnaire soon after the birth of the newborn, including child's birth order, mother's education, race/ethnicity, mother's age at child's birth and whether or not the mother smoked during pregnancy. Infant diet data were collected at 3, 6, 9, 12 and 15 months by telephone or face-to-face interview. For the previous 3 months, mothers were asked the date of introduction of all milks, formulas and foods that the infant consumed. The type of formula and cereal, as well as juice, fruit, vegetables, meat, breads, other dairy products, eggs, sweets and snack foods, were recorded. Breast feeding duration was also determined. These methods and infant diet variables have been used extensively in investigations of diabetes autoimmunity,¹⁶ coeliac disease autoimmunity¹⁷ and wheat allergy.¹⁸ At each clinic visit, the child's height and weight were measured, and body mass index (BMI; weight (kg)/height² (m²)) calculated. z Scores for the normal distribution of BMI by age were also calculated to determine how far a child's BMI was from the expected mean at that age, based on data for children in the US. For race/ethnicity, children were classified into non-Hispanic white or other. The other category included Hispanic (77.8%), African-American (6.7%), Asian (2.2%), American Indian (0.7%) and biracial (12.6%).

Data were collected prospectively to evaluate the child's exposure to tobacco smoke. At each clinic visit, questions were asked specifically about whether the mother or father of the child currently smoked, and whether the child was exposed to cigarette smoke on a regular basis (any exposure at least once per week) from anyone other than the parents. These "other" individuals were largely care givers such as baby sitters, grandparents, step-parents or family friends. These questionnaires were validated by showing that responses were significantly associated with urine cotinine concentrations, a marker of tobacco smoke inhalation.¹⁹ We developed two variables for the purposes of these analyses, one indicating parental smoking and the other indicating smoking by a care giver other than the parents.

Measurement of rheumatoid factor

At the time of this study, DAISY was following 1386 children. We identified 688 children who had a sufficient amount of available serum and whose parents had given informed consent to have their child's blood tested for autoantibodies other than those associated with T1DM. The last sample collected from each child—that is, when the child was the oldest—was selected and tested for the presence of rheumatoid factor.

Assays were performed at the University of Colorado at Denver and Health Sciences Center's Rheumatology Clinical Research Laboratory (Denver, Colorado, USA). Rheumatoid factor was measured in sera using nephelometry (Dade Behring, Newark, Delaware, USA). A child was classified as rheumatoid factor positive if he/she tested ≥ 15 IU/ml, as per the manufacturer's specifications. Twenty three of these samples were positive for rheumatoid factor, ranging from 15.2 to 138.9 IU/ml, for a prevalence of 23/688 = 3.3% (fig 1). Of the 688 children, 37 were removed because of missing data on infant diet, tobacco smoke, race/ethnicity or maternal education, resulting in 651 children in the final analyses. No rheumatological evaluation was carried out on these children; however, based on parental report, no children had been diagnosed with JRA at the time of the blood sample collection.

Data analysis

On the basis of the logistic model, and using the cohort defined above, the study could detect odds ratios (ORs) between 2.5 and 3.0 with 80% power, for exposures with 30–70% prevalence (two-sided tests, 5% significance level). All statistical analyses were performed using SAS V.9.1. Statistical analysis was performed using logistic regression for each of the study variables in both univariate and multivariate models. Variables were included in the final model if they were statistically significant based on the Wald χ^2 p value, or if their inclusion in the model altered the OR of the variable of interest by $>10\%$. Interactions were explored by including interaction terms in the models. Significance of the interactions was determined by the Wald χ^2 p value and the fit of the model, as determined by the -2 log likelihood statistic. On the basis of the results of interaction analyses, stratified multivariate analyses were conducted.

RESULTS

Rheumatoid factor-positive children were younger (mean (standard deviation (SD)) 5.33 (2.55) years), than those in the rheumatoid factor-negative group (6.43 (2.60) years; table 1). No other differences by rheumatoid factor status were observed in univariate analysis. All of the variables listed in table 1 were considered for inclusion in the multivariate model. The best-fitting model, as determined by the -2 log likelihood statistic, included age, sex, HLA-DR4 status, race/ethnicity, maternal education, breast feeding duration, exposure to tobacco smoke from a care giver other than a parent and four interaction terms, consisting of HLA-DR4 interacting with four different risk factors. The presence of these significant interaction terms strongly suggested that risk factors for autoantibody positivity differed by HLA-DR status in these children. Therefore, we performed separate analyses in those

Figure 1 Rheumatoid factor (RF) levels for 651 healthy children by age.

Table 1 Descriptive characteristics of the cohort and associations between selected environmental and perinatal factors and rheumatoid factor positivity

	RF positive	RF negative	Univariate OR (95% CI)	p Value
Race/ethnicity				
Non-Hispanic white	20 (87.0%)	496 (79.0%)		
Other*	3 (13.0%)	132 (21.0%)	0.56 (0.17 to 1.93)	0.360
Maternal education level (years)				
≤ 12	4 (17.4%)	137 (21.8%)		
>12	19 (82.6%)	491 (78.2%)	1.32 (0.44 to 3.95)	0.614
Sex				
Male	16 (69.6%)	336 (53.5%)		
Female	7 (30.4%)	292 (46.5%)	0.50 (0.20 to 1.24)	0.136
HLA genotype				
DR4 -	9 (39.1%)	181 (28.7%)		
DR4 +	14 (60.9%)	448 (71.3%)	0.63 (0.27 to 1.47)	0.281
Family history of type 1 diabetes				
No	11 (47.8%)	377 (60.0%)		
Yes	12 (52.2%)	251 (40.0%)	1.63 (0.71 to 3.77)	0.246
Age in years, mean (SD)	5.33 (2.55)	6.43 (2.60)	0.83 (0.69 to 0.99)	0.046
BMI z score, mean (SD)	-0.02 (1.13)	0.19 (1.00)	0.081 (0.52 to 1.25)	0.336
Age at introduction of cereals				
4-6 months	13 (56.5%)	320 (51%)		
Other	10 (43.5%)	308 (49%)	0.80 (0.35 to 1.85)	0.600
Breast feeding duration (months)				
0-3	12 (47.8%)	231 (36.8%)		
>3	11 (52.2%)	397 (63.2%)	0.64 (0.28 to 1.46)	0.286
Exclusive breast feeding duration† (months)				
0-3	17 (80.9%)	439 (79.2%)		
>3	4 (19.0%)	115 (20.8%)	0.90 (0.30 to 2.72)	0.849
Current parental smoking status				
No	17 (73.9%)	485 (77.1%)		
Yes	6 (26.1%)	144 (22.9%)	1.19 (0.46 to 3.07)	0.724
Exposure to cigarette smoking by care giver/relative/friend				
No	19 (82.6%)	543 (86.5%)		
Yes	4 (17.4%)	85 (13.5%)	1.35 (0.45 to 4.05)	0.598
Maternal smoking during pregnancy‡				
No	20 (90.9%)	522 (91.4%)		
Yes	2 (9.1%)	49 (8.6%)	1.07 (0.24 to 4.69)	0.933

*Other includes Hispanic (77.8%), African-American (6.7%), Asian (2.2%), American Indian (0.7%) and biracial (12.6%).

†Data on exclusive breast feeding duration were missing for 76 children.

‡Data on smoking during pregnancy were missing for 58 children.

HLA, human leucocyte antigen; RF, rheumatoid factor.

children who were positive for one or more copies of HLA-DR4 and in those who had no copies of HLA-DR4 (table 2).

Among children who had no HLA-DR4 alleles, rheumatoid factor-positive children were less likely to have been breast fed for >3 months (adjusted OR 0.18; 95% CI 0.04 to 0.99), more likely to have been exposed to smoking by a care giver other than their parents (adjusted OR 5.38; 95% CI 0.93 to 31.27), and more likely to be of an ethnic/racial group other than non-Hispanic white (adjusted OR 6.94; 95% CI 1.10 to 43.88) compared with rheumatoid factor-negative children, after adjusting for age, sex and maternal education. In children who had at least one HLA-DR4 allele, no risk factors were found to be significantly associated with being positive for rheumatoid factor (table 2).

DISCUSSION

To investigate the potential environmental associations for polyarticular JRA and rheumatoid arthritis, we examined risk factors for the intermediate phenotype of rheumatoid factor

positivity in healthy children with increased genetic risk for rheumatoid arthritis, polyarticular JRA and T1DM. We observed several significant interaction terms that suggested that the risk factors of rheumatoid factor differed by HLA-DR4 status, leading to separate investigations of environmental risk factors in DR4-positive and DR4-negative children.

In HLA-DR4-negative children, breast feeding for >3 months was associated with a decreased risk of rheumatoid factor positivity, which is consistent with a previous study that found that children with JRA were less likely to have been breast fed than controls,¹⁰ and with a study in adults that suggested that rheumatoid arthritis was less likely to develop in those who had been breast fed.²⁰ Interestingly, we did not find an association with duration of exclusive breast feeding, nor timing of introduction of cereals in the infant diet, two factors that have been shown to influence the risk of other forms of autoimmunity in children, such as diabetes¹⁶⁻²¹⁻²² and coeliac disease.¹⁷⁻²³ This suggests that the protective effect of breast feeding on rheumatoid factor positivity may be due to the

Table 2 Perinatal and early childhood risk factors for rheumatoid factor positivity stratified by HLA DR4 status

	DR4– 9 RF+ and 180 RF– children		DR4+ 14 RF+ and 431 RF– children	
	OR (95% CI)	p Value	OR (95% CI)‡	p Value
Age*	0.71 (0.46 to 1.09)	0.115	0.88 (0.71 to 1.10)	0.259
Other race/ethnicity†	6.94 (1.10 to 43.88)	0.039		
Male	0.23 (0.04 to 1.41)	0.112		
Maternal education ≤12 years	4.91 (0.43 to 56.48)	0.202		
Breast feeding duration >3 months	0.18 (0.04 to 0.99)	0.049		
Exposure to cigarette smoking by care giver/relative/friend	5.38 (0.93 to 31.27)	0.061		

RF, rheumatoid factor.

*Odds ratio calculated for a 1-year increase in age.

†Other includes Hispanic (70.0%), African-American (16.7%), Asian (3.3%) and biracial (10.0%), compared with non-Hispanic white.

‡Race, sex, maternal education, breast feeding duration and other environmental tobacco smoke did not add significantly to the model in DR4 positive children, and were removed.

immune-modulating effects of breast milk, including large amounts of immunoglobulin (Ig)A and antigen tolerance capabilities, rather than to a delay in the introduction of foreign dietary antigens. We also found that having regular exposure to a person other than a parent who smokes increases the risk of rheumatoid factor positivity in children who are HLA-DR4 negative, which is consistent with the observation that current parental smoking was more prevalent in a group of children diagnosed with JRA.¹⁴ The reason for the observed increased risk with being around a care giver who smokes, but not with being around a parent who smokes is not clear, but it may be due to the way the question is phrased and to the relative amount of exposure. Conceivably, a child may spend more time with the person who is taking care of it during the day, while the parents are at work. Thus, they may have a greater chance of exposure to tobacco smoke than if the parents smoke. Also, it is possible that parents may make an effort to avoid smoking around their child. The questions regarding the parents smoking are worded in a slightly different way, which may have accentuated this difference. For example, when we ask whether the parents “currently smoke”, we never specifically ask if the parents smoke in front of the children, whereas the other question is phrased to pick up whether “the child is exposed to cigarette smoke on a regular basis ... from anyone other than the parents”, which may represent a more intense exposure. As to what factor in cigarette smoke may lead to rheumatoid factor production, in adult rheumatoid arthritis models it is postulated that tobacco smoke may initiate the autoimmune response by stimulating B cells directly,²⁴ resulting in increased levels of rheumatoid factor.

Breast feeding duration and exposure to tobacco smoke were not associated with rheumatoid factor positivity in the DR4-positive children. The lack of environmental risk factors in the group with HLA-DR4 may possibly be related to a multi-hit aetiology, where those without the genetic susceptibility marker need environmental exposures to convert to autoantibody positivity whereas those who already have a genetic susceptibility marker do not need these exposures to become positive.

Another interesting finding in this cohort is that HLA-DR4 was not associated with rheumatoid factor positivity in healthy children. This finding is similar to that reported by others who have recently found that HLA seems to be associated with anti-cyclic citrullinated-peptide positive rheumatoid arthritis rather than with rheumatoid factor.²⁵ Although tobacco smoke exposure has been associated with the development of rheumatoid arthritis and rheumatoid factor positivity and,

more recently, strongly associated with the presence of anti-cyclic citrullinated peptide in HLA DR4-positive patients with rheumatoid arthritis,²⁶ on the basis of our finding of passive tobacco smoke exposure and rheumatoid factor in HLA-DR4-negative children, there may be another genetic association besides HLA-DR4 that may be responsible for the link between smoking and rheumatoid factor production. In support of this, a recent study suggests that exposure to tobacco smoke increased the risk of rheumatoid arthritis among older Caucasian women who were not positive for the HLA-DRB1 shared epitope, which includes HLA-DR4, and is the best known genetic risk factor for rheumatoid arthritis; however, tobacco exposure did not increase the risk of disease in those positive for the shared epitope.²⁷

A limitation of this study is that no physical examination was performed on these children to determine whether they had clinically relevant disease in addition to rheumatoid factor autoantibodies. Figure 1 shows the range of rheumatoid factor levels in the children. Although some of the positive levels were near the cut-off of 15 IU/ml, half were >20 IU/ml and one third at ≥40 IU/ml. A positive rheumatoid factor level in this study was used as an intermediate phenotype for JRA and rheumatoid arthritis based on levels in adults. It is unclear what a rheumatoid factor positive level truly is in children, as there are no population data, to our knowledge. Thus, using the adult level as a cut off may not be a true representation of a positive level in children. There was also no follow-up for disease or worsening of autoimmunity, although this is something we would like to pursue. Small numbers within some of the groups resulted in marginal associations, so an association of a small effect could have been missed. Our study population was at increased risk of rheumatoid arthritis and polyarticular JRA based on HLA-DR4 prevalence, which limits the generalisability of our results to the general population. A major strength of this study is the prospective data collection, which increased accuracy and eliminated recall bias because all exposures were assessed before the measurement of the outcome. In addition, the use of an autoantibody-negative comparison group from within the DAISY cohort reduced the possibility of selection bias.

These findings indicate that environmental influences may affect children's immune responses as evidenced by rheumatoid factor positivity. An additional follow-up of this and other cohorts of children and adults is necessary to elucidate whether these influences result in clinical diseases such as polyarticular JRA and seropositive rheumatoid arthritis.

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