Mothers' Experiences of Serious Life Events Increase the Risk of Diabetes-Related Autoimmunity in Their Children

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OBJECTIVE — Stressful life events have been shown to constitute a risk factor for type 1 diabetes during childhood. Our aim was to investigate in the general child population (i.e., irrespective of genetic risk for type 1 diabetes) whether mothers' experiences of serious life events, such as divorce and violence, were associated with diabetes-related autoimmunity in their children at age 2.5 years.

RESEARCH DESIGN AND METHODS — The study cohort was comprised of the first 5,986 consecutive children and their families from the prospective population-based All Babies in Southeast Sweden project for whom 2.5-year study data were available. Data were drawn from parental questionnaires that included questions about experiences of serious life events and the blood samples taken from the children when the children were age 2.5 years. The blood samples were analyzed for diabetes-related autoantibodies against tyrosine phosphatase and GAD.

RESULTS — Mothers' experiences of divorce (odds ratio 3.6, 95% CI 1.4–9.6, $P < 0.05$) and violence (2.9, 1.0–7.8, $P < 0.05$) were associated with diabetes-related autoimmunity in the children, independent of a family history of type 1 diabetes.

CONCLUSIONS — The results support the β-cell stress hypothesis and suggest that maternal experiences of serious life events such as divorce and violence seem to be involved in the induction or progression of diabetes-related autoimmunity in children at age 2.5 years, independent of family history of type 1 diabetes.

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Serious life events have been retrospectively investigated in children newly diagnosed with type 1 diabetes and are considered to be a risk factor for type 1 diabetes (1,2). Thernlund et al. (1) found that negative life events, such as divorce, serious illness within the family, or loss of a first-degree relative, during the first 2 years of life increased the risk of type 1 diabetes. Another Swedish case-control study found that stressful life events related to actual or threatened loss within the family were associated with the onset of manifest type 1 diabetes (2). We recently reported that psychological stress, including experiences of unspecified but serious life events, were associated with the induction or progression of diabetes-related autoimmunity in infancy in the general population (3).

It has been suggested that mothers' experiences of serious life events, such as divorce or violence, may constitute psychological stress for their children. Bowlby's attachment theory begins with the observation that infants in the world of our evolutionary ancestors needed to maintain physical proximity with their caregivers to survive. Such proximity was supported by the evolution of a biologically based adaptation to parental moods, signals, and behaviors found in the range of likely caregiving environments (4,5). Ainsworth and collegues (6,7) suggested, and it is now widely accepted within attachment theory, that small children are biologically predisposed to use their caregiver as a "secure base" from which to explore the environment. When the child feels threatened or distressed, he or she will turn to the secure base for protection and comfort. Therefore, any restriction of a child's access to the secure base when the child is afraid, upset, or in pain is stressful for the child during his or her first years.

Divorce is a disruption in the family triad that normally includes repeated or prolonged separations from one or both of the primary caregivers; these separations may disrupt the child's access to his or her secure base. Furthermore, mothers who have been victims of violence and have not achieved psychological resolution about the event are more likely to act in frightening or frightened ways toward their own child, thus placing the child in an unsolvable situation of biologically needing to approach the mother who at the same time is the source of the alarm (8). Serious diseases or accidents within the family and loss of relatives are other life events that may affect the child negatively, even if they do not represent a threat to the child's secure base. For a parent to become unemployed may be stressful and could presumably negatively affect the family climate.

Type 1 diabetes is likely caused by an autoimmune destruction of the insulin-producing β-cells in the pancreas (9). The β-cell stress hypothesis (3,10,11), an extension of the accelerator hypothesis (12), suggests that a number of different factors (including lifestyle factors such as psychological stress and rapid weight gain) could cause an increase in insulin demand, thereby leading to β-cell stress, which in turn might lead to an autoimmune reaction, particularly in genetically predisposed individuals. Therefore, different sources of psychological stress, including serious life events, should be

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Abbreviations: ABIS, All Babies in Southeast Sweden; GADA, autoantibody to GAD; IA-2A, autoantibody to tyrosine phosphatase.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

investigated as risk factors for diabetes-related autoimmunity and type 1 diabetes.

The best marker of diabetes-related autoimmunity is a combination of positive concentrations of autoantibodies toward tyrosine phosphatase (IA-2As) and GAD (GADAs) (13). A positive concentration of just IA-2As alone is considered the second-best marker (14). However, positive concentrations of these diabetes-related autoantibodies at age 2.5 years represent only a slightly increased risk of developing manifest type 1 diabetes.

Our aim was to investigate whether psychological stress, measured as experiences of serious life events, is associated with diabetes-related autoimmunity early in life and thus might constitute a risk factor for the development of type 1 diabetes. The current study was undertaken prospectively in the general child population (i.e., regardless of genetic risk for type 1 diabetes), which is an advance over existing retrospective studies and studies searching for risk factors in samples with a high genetic risk for type 1 diabetes. We were especially interested in divorce and maternal experiences of violence because of their possible threat to the child's secure base. We were also interested in other negative life events that may have a negative impact on the family climate. Therefore, the mothers' experiences of serious life events were assessed and analyzed in relation to positive concentrations of diabetes-related autoantibodies in their children aged 2.5 years; an association was expected.

A number of external factors that might influence the association between psychological stress and the occurrence of diabetes-related autoantibodies were also investigated, namely, diabetes in the family or the extended family, increased parental age (15–17), childhood infections (18,19), body weight of the child (12), delivery by cesarean section (16), and the need for neonatal intensive care (20).

RESEARCH DESIGN AND METHODS

From 1 October 1997 to 1 October 1999, all parents-to-be in southeast Sweden were asked to participate in the All Babies in Southeast Sweden (ABIS) project; 78.6% agreed, yielding a sample of ~17,000 families at birth, of which 16,070 completed the at-birth questionnaire, ~10,700 participated in the 2.5-year follow-up, and 8,805 completed the 2.5-year questionnaire. In the current study, the 2.5-year data, including mothers' responses concerning recent life events and blood samples from the children, were examined in the first 5,986 consecutive families for whom 2.5-year data were available for statistical analyses (i.e., there was no specific selection). Due to incomplete questionnaires, there was an internal drop out of <1.2% in the variables assessing serious life events. In the sample, there was a slight underrepresentation of parents with a low level of education and foreign-born parents compared with the general population of the Östergötland province in Sweden (21) where data were collected. Further details concerning the ABIS sample have been published elsewhere (22).

In striving to assess the mechanisms involved in the onset of autoimmunity, it was of interest to investigate recent serious life events. Data concerning divorce and autoantibody status (assessed in whole blood drawn from the children at age 1 year) were available from the 1-year follow-up so the association between divorce and autoimmunity could be longitudinally investigated. The longitudinal follow-up sample included 1,845 children who had not experienced divorce and did not have autoantibodies at age 1 year.

Capillary blood was drawn from the children and a questionnaire was given to the parents at the 2.5-year check-up at the well-child clinics. No reminders were used. The capillary tubes with whole blood were analyzed at the Clinical Research Centre, Faculty of Health Sciences, Linköping.

Measures

Maternal experiences of serious life events since the birth of the child (i.e., within the previous 2.5 years) were assessed with one yes/no question and seven structured follow-up questions to determine the specific type of serious life event. The seven choices of events were becoming divorced, being subjected to violence, experiencing a serious disease in the family, experiencing a serious accident in the family, losing a relative, becoming unemployed, and having a spouse or common-law spouse become unemployed.

IA-2As and GADAs were assessed in whole blood drawn from the children at age 2.5 years. The blood samples were analyzed by an immunoprecipitation method, as described by Savola et al. (23). Positivity for IA-2As and GADAs was determined as having antibody concentrations >95th percentile for 2.5-year-old healthy children, which corresponds to 26.0 World Health Organization units for IA-2As and 82.2 units for GADAs. We observed that 304 children were positive for IA-2As and 291 were positive for GADAs. Positivity for IA-2As and GADAs was also combined to give single/double positivity because signs of diabetes-related autoimmunity were considered to be as important as specific autoantibody positivity. Single/double positivity was defined as having positive concentrations of either IA-2As or GADAs ($n = 531$) or both ($n = 32$); thus 563 children were single/double positive. No analyses of double positivity were done due to the limited number of children being positive for IA-2As as well as GADAs ($n = 32$).

In accordance with the Third International Workshop (Diabetes Autoantibody Standardization Program, 2003), our specificity was 100 and 98% and our sensitivity 48 and 78% for IA-2A and GADA assays, respectively. The intra-assay coefficient of variation was 5.2%, and the interassay coefficient was 13%.

External factors that might influence the association between psychological stress and the occurrence of diabetes-related autoantibodies (15–19) were assessed in the questionnaires. Type 1 diabetes in the family was defined as the child's mother, father, or sibling having type 1 diabetes, whereas type 1 diabetes in the extended family included maternal and paternal grandparents of the child as well. Maternal and paternal ages at the child's birth were assessed. Two different measures of childhood infections were assessed at age 1 year: the number of episodes of gastroenteritis and the number of infections treated with penicillin. Both were dichotomized into three episodes or more versus two or less. The body weight of the child was assessed as the child's size for gestational age at birth and BMI at ages 1 and 2.5 years. Size for gestational age was calculated according to the formula of the National Swedish Board of Health and Welfare, yielding the categories small, appropriate, or large for gestational age. Size for gestational age was dichotomized into normal versus small or large for gestational age, as both small and large for gestational age may increase the risk for β-cell stress compared with normal size for gestational age. BMI was calculated based on the height and weight measured at the well-baby clinic at ages 1 and 2.5 years, and two groups (above and below the 90th percentile) were formed. Delivery mode was assessed at birth and coded

Table 1—*Association of different types of serious life events with concentrations of 1A-2A and GADA and single/double-autoantibody positivity in children age 2.5 years*

		IA-2As (n = 304)			GADAs (n = 291)			Double/single positivity (n = 563)		
	n*	OR (95% CI)	χ^2	P	OR (95% CI)	χ^2	P	OR (95% CI)	χ^2	P
Experienced a serious life event	1,396	1.1 (0.9–1.5)	0.627	0.428	1.1 (0.8–1.4)	0.122	0.727	1.1 (0.9–1.3)	0.683	0.409
Divorced	186	1.8 (1.1–3.1)	5.130	0.024	0.5 (0.2–1.3)	1.963	0.161	1.1 (0.7–1.9)	0.419	0.517
Subjected to violence	22	1.8 (0.4–7.9)	F†	0.316	3.1 (0.9–10.7)	F†	0.090	2.9 (1.1–7.8)	F†	0.049
Serious disease in the family	315	1.2 (0.7–2.0)	0.607	0.436	0.9 (0.5–1.5)	0.170	0.680	1.1 (0.8–1.6)	0.236	0.627
Serious accident in the family	55	1.1 (0.3–3.5)	F†	0.758	0.7 (0.2–3.0)	F†	1.000	1.0 (0.4–2.4)	0.005	0.941
Lost a relative	736	1.0 (0.7–1.5)	0.014	0.905	1.1 (0.8–1.6)	0.491	0.483	1.1 (0.8–1.4)	0.154	0.695
Became unemployed	101	1.4 (0.6–3.1)	0.787	0.375	0.9 (0.4–2.4)	0.007	0.936	1.3 (0.7–2.4)	0.756	0.384
Spouse/common-law spouse became unemployed	54	2.0 (0.8–5.1)	F†	0.188	0.7 (0.2–3.1)	F†	1.000	1.4 (0.6–3.2)	0.823	0.346

Data are bivariate ORs (95% CIs) and χ^2associations. Double/single positivity: positive for IA-2As and/or GADAs. *Number of cases in the category; †Fisher's exact test was used because the expected count was <5 in 25% of the cells.

as normal delivery versus delivery by cesarean section; all other types of problematic deliveries were excluded. The need for neonatal intensive care was assessed at birth.

Statistical analyses

SPSS 11.5 was used to calculate χ^2 values, odds ratios (ORs) with 95% CIs, and Student's t tests. ORs were used as approximate measures of relative risk. In all t tests, Levene's test was used to investigate the equality of variance, and the degrees of freedom were adjusted when equal variance could not be assumed.

The association with a number of external factors was investigated; if they were not related to experiences of serious life events, they were not likely to explain our findings. External factors associated with the experience of serious life events were analyzed concerning their association with positive concentrations of autoantibodies (IA-2As, GADAs, and single/double-autoantibody positivity); if they were not related to autoantibodies, they were not likely to explain the results.

Ethical considerations

The parents gave their consent after receiving oral and written information about the study as well as after being given the opportunity to see a video about the project. Active return of a completed at-birth questionnaire and/or biological samples (in addition to routinely collected cord blood) was considered as informed consent. The parents were not automatically informed of their child's autoantibody status and <1% actively requested this information.

The ABIS project and the current study were approved by the Research Ethics Committees of the Faculty of Health Science at the University of Linköping, Sweden, and the Medical Faculty at the University of Lund, Sweden.

RESULTS

— Our results showed that 23% of the mothers had experienced some kind of negative life event since their child's birth (i.e., during the previous 2.5 years): 3%, divorce; 0.4%, violence; 5%, serious disease in the family; 1%, serious accident in the family; 12%, loss of a relative; 2%, becoming unemployed; and 1%, having a spouse or common-law spouse who became unemployed.

A significant association between the experience of divorce and positive concentrations of IA-2As was found (Table 1). The association remained in the longitudinal follow-up sample (n = 1,845), among whom 12.5% (n = 5) of the children exposed to divorce (n = 40) had developed autoimmunity compared with 3.8% (n = 68) of those not exposed to divorce (n = 1,805) by Fisher's exact test ($P < 0.05$) (data not shown in Table 1, where only results concerning n = 5,986 are presented). The OR for autoimmunity after divorce was 3.6 (95% CI 1.4–9.6) in the longitudinal follow-up sample. Mothers' experiences of violence were significantly associated with single/double-autoantibody positivity and approached significance with positive concentrations of GADAs (Table 1).

An examination of the ORs in Table 1 shows that there is a trend of increased risk concerning positive IA-2A concentrations and single/double positivity associated with almost all of the life events measured, even though most of them did not reach statistical significance.

A number of different external factors were investigated concerning divorce and maternal experiences of violence. Parents who were divorced were younger than nondivorced parents, but the mean difference was only 1 year (Table 2). The association between parental age and divorce did not remain in the longitudinal follow-up sample (maternal age: $t[41] = -1.167$, NS; paternal age: $t[40] = -0.325$, NS), nor was parental age associated with IA-2A positivity in the complete sample (n = 5,986) (maternal age: $t[5,684] = -0.239$, NS; paternal age: $t[5,657] = -0.322$, NS). The existence of type 1 diabetes in the extended family was associated with whether the mother had experienced violence (Table 2); however, it was not associated with single/double-autoantibody positivity [$\chi^2(1) = 0.377$; NS] or GADA positivity [$\chi^2(1) = 0.149$; NS]. There was a trend that approached significance for a BMI >90th percentile at age 2.5 years to be associated with maternal experiences of violence (Table 2). However, BMI at age 2.5 years was not associated with IA-2As [χ^2 (1) = 1.310; NS], GADAs [$\chi^2(1) = 0.572$; NS], or single/double-autoantibody positivity [$\chi^2(1) = 0.001$; NS]. These findings indicated that none of the external factors investigated were likely to explain the association between experiences of certain serious life events and autoimmunity in the child.

CONCLUSIONS — The current results lend initial support to the β-cell stress hypothesis (3,10,11) and are in line with our earlier finding that psychological stress may be involved in the induction or progression of diabetes-related autoimmunity in infants (3). The risk of having developed autoimmunity at age 2.5 years after having been exposed to a parents' divorce was approximately threefold. Mothers' experiences of violence also increased the risk of development of autoimmunity in children by about threefold. These associations were found despite a slight underrepresentation of low education and foreign origin in the sample.

The association between mothers' experiences of serious life events and the development of diabetes-related autoimmunity in their children could be explained by transferred psychological stress, although the current study design only permitted us to speculate about the mechanisms by which this might occur. However, children are very sensitive to parental moods, signals, and behaviors (4), and it has been shown that maternal stress during infancy may sensitize children to later stress exposure (24). Divorce and maternal experiences of violence may be two of the most important sources of early stress due to the threat concerning access to the secure base and the risk of an unsolvable approach-avoidance paradox, respectively. The child's situation is of course even more complicated if the violent acts toward the mother are committed by the father (no data were available on the identity of the perpetrator in our study).

Serious diseases, serious accidents, the loss of relatives, and unemployment did not significantly increase the risk of diabetes-related autoimmunity in the children, although our results did point in that direction. It is possible that our failure to find these associations was a result of the fact that we assessed the mother's, rather than the child's, experience of serious life events. For example, the person lost was probably one of the mother's parents (i.e., grandparent to the child) and not a first-degree relative of the child. Young children will likely sense some of the parent's sadness concerning a loss, but a loss could also bring about a period of increased family closeness with more emotional affection. This reasoning may also apply for other life events such as diseases and accidents in the family. The mothers' experiences of positive life events were not assessed, as it is less likely

Table 2—*Associations among divorce, maternal experiences of violence, and external biological factors*

		Divorce				Violence			
	*n**	Yes	No	Statistical analyses	*P*	Yes	No	Statistical analyses	*P*
Distribution in the categories		186 (3.1)	5,735 (96.9)	—	—	22 (0.4)	5,893 (99.6)	—	—
Presence of type 1 diabetes									
In family	128	3.1	96.7	$\chi^2(1) = 0.000$	1.000	0.8	99.2	F†	0.383
In extended family	517	3.7	96.3	$\chi^2(1) = 0.530$	0.466	1.0	99.0	F†	0.038
Mother's age (years)	—	28.7 ± 5.3	29.8 ± 4.5	$t(194) = -2.794$	0.006	29.2 ± 6.7	29.8 ± 4.5	$t(21) = -0.424$	0.676
Father's age (years)	—	31.3 ± 5.9	32.2 ± 5.2	$t(190) = -2.025$	0.044	31.6 ± 7.6	32.2 ± 5.3	$t(5748) = -0.489$	0.625
Childhood diseases during 1st year of life									
Gastrointestinal influenza ≥3 times	32	6.3	93.8	F†	0.233	0.0	100	F†	1.000
Infections treated with penicillin ≥3 times	264	1.9	98.1	$\chi^2(1) = 1.278$	0.258	0.8	99.2	F†	0.302
Small or large size for gestational age at birth	387	3.6	94.6	$\chi^2(1) = 0.233$	0.629	0.5	99.5	F†	0.643
BMI >90th percentile									
At age 1 year	453	3.5	94.5	$\chi^2(1) = 0.585$	0.444	0.9	99.1	F†	0.097
At age 2.5 years	573	3.0	97.0	$\chi^2(1) = 0.000$	0.997	0.9	99.1	F†	0.055
Delivery by cesarean section	667	3.0	97.0	$\chi^2(1) = 0.062$	0.803	0.0	100	F†	0.156
Need for neonatal intensive care	501	3.2	96.8	$\chi^2(1) = 0.000$	0.995	0.2	99.8	F†	1.000

Data are *n* (%), %, or means ± SD. Bivariate χ^2 associations are shown. *Number of cases in category; †Fisher's exact test was used because the expected count was <5 in 25% of the cells.

that they would have any specific impact on the child's physiological stress level.

Psychological stress of different kinds has been shown to influence the immune response (25) and has recently been associated with the development of allergies (26). Psychological stress is also known to increase insulin demand and decrease insulin sensitivity, which could lead to β-cell stress and an autoimmune reaction, particularly in genetically predisposed individuals. However, based on the design of the current study, it is premature to speculate on the biological links responsible for the associations found.

In the present study, different kinds of serious life events were considered markers of psychological stress as they affect the family climate in a negative way, which in turn may induce stress in the child. Thus the theoretical association found was really between stress and β-cell autoimmunity, of which IA-2As and GADAs are relevant markers. Therefore, we cannot explain why divorce was associated with IA-2As whereas maternal experiences of violence were associated with single/double-autoantibody positivity and almost significantly associated with GADAs.

Insulin autoantibodies often appear as the first sign of β-cell autoimmunity in children and, especially in young children, are associated with type 1 diabetes. In the current study, β-cell autoantibodies were analyzed in lysed capillary blood, in which the analysis of insulin autoantibodies is not recommended due to high unspecific binding (data not shown). Thus the results so far are restricted to the emergence of IA-2As and GADAs.

Levels of diabetes-related autoantibodies fluctuate during early childhood. Despite this fact, positive concentrations of IA-2As and GADAs are still considered to be the best markers of type 1 diabetes (13). Most of the children in our sample with positive concentrations will probably never develop diabetes, and the occurrence of autoantibodies at age 2.5 years can be interpreted as representing only a slightly increased risk of developing manifest type 1 diabetes. The ultimate aim of the ABIS project is to determine the importance of different stress factors for the development of manifest type 1 diabetes, but for this purpose a longer follow-up time is needed when an unselected population of children is studied prospectively. However, it is still noteworthy that mothers' experiences of negative life events, such as divorce and violence, seem to put some children at risk for the development of a serious and lifelong disease.

The results of the current study also support those of earlier retrospective studies in that experiences of serious life events might constitute a risk factor for type 1 diabetes. Hägglöf et al. (2) found that actual loss or threat of loss in the family the year before diagnosis was overrepresented among children with newly diagnosed type 1 diabetes compared with matched controls. We would like to suggest that mothers' experiences of negative life events, such as divorce and violence, could actually be severe enough to negatively affect their children's insulin sensitivity or immune response. Our results are also in line with those of Thernlund et al. (1), suggesting that exposure to serious life events during the first years of life constitutes a risk factor for type 1 diabetes. In fact, our results suggest that psychological stress might be involved in the induction or progression of the diabetes-related autoimmune β-cell destruction purported to precede manifest type 1 diabetes. Psychological stress has previously been specifically related to the induction or progression of diabetes-related autoimmunity rather than to an enhanced general immune response, as reflected by the lack of an association between stress factors and antibodies toward tetanus toxoid (3).

No correction for multiple statistical analyses was used for the 28 χ^2 analyses conducted. Therefore, it is important to note that the pattern of results in large part confirm our a priori hypotheses. The results are also of exploratory value as this was the first prospective study of its kind.

Type 1 diabetes is considered to be a multifactorial disease; in this study, a number of factors that might influence the association between psychological stress and the occurrence of autoantibodies were investigated. According to our analysis, none of the following known risk factors of type 1 diabetes were likely to explain the current results: type 1 diabetes in the family or the extended family, increased parental age (15–17), childhood infections (18,19), body weight of the child (12), delivery by cesarean section (16), and the need for neonatal intensive care (20).

In conclusion, our results lend initial support for the β-cell stress hypothesis (3,10,11) and suggest that mothers' experiences of serious life events, such as divorce and violence, could be involved in the onset or progression of the diabetes-related autoimmune β-cell destruction and not only the precipitation of manifest type 1 diabetes, as earlier shown.

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