




Prenatal and early life factors and type 1 diabetes

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Abstract

Background The prevalence of type 1 diabetes is increasing worldwide, suggesting that unknown environmental factors are becoming increasingly important in its pathogenesis.

Aim The aim of the study was to investigate the possible role of a number of prenatal and perinatal factors in the aetiology of type 1 diabetes.

Methods Mothers of patients diagnosed with type 1 diabetes (cases) and mothers of children born on the same day and of the same sex as type 1 diabetes patients (controls) were interviewed on a number of prenatal and perinatal factors of interest.

Results Hand washing prior to eating, frequency of bathing and total stress score were found to be positively associated with the development of type 1 diabetes on univariate analyses. Hand-washing prior to eating and frequency of house cleaning were independently associated with an increased risk of type 1 diabetes, whilst getting dirty was associated with a reduced risk in multivariate analyses. There was no association of type 1 diabetes to removing of outdoor shoes indoors or to the age of first attendance to school or pre-school. There were also no significant associations to parental smoking, parental age, birth order, infant feeding, antibiotic use, mode of delivery or birth weight.

Conclusion Our data suggest that factors that affect the skin or gut microbiome might be more important than infections or factors affecting the microbiome at other sites.

Keywords Type 1 diabetes · Environmental factors · Hygiene hypothesis · Microbiome · Psychological stress

Highlights

- The incidence of type 1 diabetes is increasing world-wide for reasons which are incompletely understood.
- The present study investigates the possible role of early life factors.
- Hand washing prior to eating, frequency of bathing and total stress score were found to be positively associated with the development of type 1 diabetes on univariate analyses. Hand-washing prior to eating and frequency of house cleaning were independently associated with an increased risk of type 1 diabetes, whilst getting dirty was associated with a reduced risk in multivariate analysis.
- Our data suggest that factors that affect the skin or gut microbiome might be more important than infections or factors affecting the microbiome at other sites.

Introduction

The incidence of type 1 diabetes is increasing world-wide in both adults [1] and children [2]. Type 1 diabetes is caused by

autoimmune destruction of pancreatic β -cells resulting in greatly diminished capacity to produce and secrete insulin [3]. This results in undetectable or very low levels of insulin, the latter being referred to as microsecretors [4]. It is estimated that 149,500 new cases <20 years of age were diagnosed in 2021 [5], with over 1.1 million persons currently living with type 1 diabetes globally [6]. Type 1 diabetes is associated with increased mortality and decreased life expectancy. Analysis of registry data in Scotland has revealed the age-adjusted incidence rate ratio of first cardiovascular (CV) event and of all-cause mortality associated with T1DM compared to the non-diabetic population to be

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3.0 (95% confidence intervals, CI: 2.4–3.8) and 2.7 (95% CI: 2.2–3.4) respectively [7] in women. In men the age-adjusted incidence rate ratio for first CV event and all-cause mortality were 2.3 (95% CI: 2.0–2.7, $p < 0.001$) and 2.6 (95% CI: 2.2–3.0) respectively [7]. The number of years lost in 20-year-old men ranges from 29.3 years to 50.6 years and 35.0–53.9 years in 20-year-old women, depending on risk factor values, HbA_{1c} and estimated glomerular filtration rate [8]. Type 1 diabetes is also associated with significant health and societal cost; the difference in lifetime costs has been estimated at \$813 billion in the United States [9].

The findings that type 1 diabetes is increasing worldwide [1, 2] and that the role of genetic factors is decreasing with time [10] suggest that environmental factors are playing an increasingly important role in the aetiology of type 1 diabetes. Type 1 diabetes is known to have a long preclinical phase with islet cell auto-antibodies appearing in the early years of life [11, 12]. These observations suggest that environmental factors in the first few years of life are likely to be important. Early age of onset is also associated with more aggressive disease and higher mortality [13], again highlighting the importance of early life factors.

Possible environmental factors include microbial exposure which can affect the host microbiome [14, 15]. Subjects with type 1 diabetes are known to exhibit differences in composition of the intestinal microbiome and a decrease in its diversity [16]. Early life factors which are known to affect the gut microbiome include infant feeding, mode of delivery, hygiene practices and parental smoking [16, 17]. Gestational age and birth weight have also been implicated as risk factors in type 1 diabetes [18] through undetermined mechanisms.

We therefore sought to investigate which early life factors are associated with type 1 diabetes. We investigated a number of early life behavioural factors which alter microbial exposure, including hygiene practices and exposure to pets. The former included ‘getting dirty’ (defined as playing in the soil and/or in dusty areas outdoors), frequency of bathing, removal of outdoor shoes indoors, hand-washing prior to eating and cleaning of the house. We also gathered data on maternal use of antibiotics during pregnancy because of its potential effect on the infant’s microbiome as well as on perinatal factors known to affect type 1 diabetes risk [16]. We also gathered data on gestational age, birth weight, mode of delivery, infant feeding, the number of household siblings, parental smoking and parental age as these may be potential confounders. Data on stress was also captured in view of its potential role in type 1 diabetes risk.

Methods

In this retrospective case-control study, mothers of patients diagnosed with type 1 diabetes who were older than 16

years of age at the time of the study were invited to participate as cases whilst mothers of children born on the same day and of the same sex of type 1 diabetes patients were invited to participate as controls. Diagnosis of type 1 diabetes was based history of diabetic ketoacidosis and/or positive antibodies to islet cell and/or glutamic acid decarboxylase and/or insulin. Controls were individuals of the same sex who were born on the same day of type 1 diabetes patients as identified from the national birth register.

Mothers of type 1 diabetes patients and of controls were invited to an interview. Written informed consent was obtained.

Data collection included date of birth of both parents, date of diagnosis of type 1 diabetes, smoking history of the mother and father prior to conception and during pregnancy, antibiotic use during pregnancy, mode of delivery, birth weight, gestational age at delivery, birth order, initial nutrition of the infant (whether breastfed or formula fed, the duration of exclusive breastfeeding and total duration of breastfeeding, timing of introduction of formula, cow’s milk and solid foods), age at first attendance to school or nursery, household and personal hygiene, exposure to pets and the number of siblings until diagnosis. Hygiene practices assessed included removal of outdoor shoes indoors, ‘allowed to get dirty’ (defined as playing in the soil and/or in dusty areas outdoors), hand-washing prior to eating and the frequency of bathing. The behavioural habits refer to the time period up to the time of diagnosis of type 1 diabetes and up to the same age for each of the controls (who were age-matched to patients).

Mothers of type 1 diabetes patients and controls were also asked whether the child experienced any life events up to the date of diagnosis of type 1 diabetes which might have caused any psychological stress in the child and to specify the nature of these life events if present. These life events were subsequently rated on the Holmes and Rahe Non-Adult Stress Scale [19] to calculate the total stress score for both type 1 diabetes patients and controls.

The study protocol was approved by the University of Malta Research Ethics Committee, the Data Protection Officer at Mater Dei Hospital and the Data Protection Commissioner.

Statistical methods

Statistical analysis was performed using SPSS. The statistical significance of differences between the 2 groups was assessed using two-tailed unpaired Student’s *t* test for normally distributed continuous variables and by the Mann–Whitney test for non-normally distributed continuous variables. The ‘z’ test was used to assess the statistical significance of differences in proportions. Statistical

significance was set at $\alpha = 0.05$. The chi square test was used to assess the statistical significance of differences in proportions in multiple groups. Logistic regression was used to investigate the independent determinants of type 1 diabetes. Sample size was determined so as to have 95% statistical power to detect statistical difference at Cohen's effect size (d) of 0.5 and 85% statistical power to detect an effect size (f^2) of 0.1 in multivariate analysis with 6 covariates at $\alpha = 0.05$.

Results

Mothers of 89 unrelated type 1 diabetes patients (48 males and 41 females) and mothers of 89 controls, matched for sex and date of birth, were included in the study. The results are summarised in Table 1. The median (interquartile range, IQR) age of type 1 diabetes patients and of controls at the time of the study was 23 years (19–27), while the median (IQR) age at diagnosis of type 1 diabetes was 11 years (7.8–14.5). Eleven patients and none of the controls had a family history of type 1 diabetes amongst first-degree relatives; 38 patients and 31 controls had a family history of other autoimmune conditions amongst first-degree relatives.

Mothers of type 1 diabetes children and controls were asked whether their children washed their hands prior to eating always, often, sometimes or never and whether their children bathed more than once daily, once daily, twice weekly, once weekly or less than once weekly. Our results show that type 1 diabetes patients washed their hands prior to eating more often than controls, the difference being statistically significant at a p value of 0.001 (Fig. 1). Type 1 diabetes patients and controls also differed significantly in their frequency of bathing (Fig. 2), whereby type 1 diabetes patients bathed more often than controls ($p = 0.008$). With regard to household hygiene, type 1 diabetes patients and controls showed no differences in the frequency of cleaning of the house (median 2.5 times weekly, IQR once weekly – once daily, for both mothers of type 1 diabetes patients and controls), in taking off their outdoor shoes indoors (42% of mothers of type 1 diabetes patients compared to 39.8% of mothers of controls, $p = 0.76$) and in the children's exposure to household pets (48.9% of type 1 diabetes patients compared to 53.4% of controls, $p = 0.55$). The proportion of mothers who allowed their children to get dirty was numerically smaller in type 1 diabetes patients (36.4%) than controls (48.9%) but this was not statistically significant ($p = 0.09$).

There was no statistically significant difference in the age at which children first attended school or nursery between type 1 diabetes patients and controls, with both groups having a median (IQR) age of first attendance to school or nursery, whichever came first, of 36 (36–36) months,

$p = 0.354$). Similarly, there was no significant difference in whether they first attended school or nursery during their first, second, third or fourth year ($p = 0.269$).

There was no significant difference between mean maternal age at delivery of type 1 diabetes patients (29.3 ± 4.6 years) and that of controls (29.1 ± 5.3 years) ($p = 0.63$). Comparing age at delivery stratified by age intervals revealed a highly statistically significant difference in the age distribution between mothers of type 1 diabetes patients and mothers of controls ($p = 0.0001$), with the maternal age at delivery of type 1 diabetes patients peaking at the 25–29 year age group while maternal age at delivery of controls peaked later at the 30–34 year age group. This difference was not observed for paternal age at delivery ($p = 0.15$). With regards to mode of delivery 16.9% of type 1 diabetes patients and 18.0% of controls were born via Caesarian Section ($p = 0.2$). There was no significant difference in the birth order of type 1 diabetes patients and controls ($p = 0.96$) and in the median number of siblings in the household until diagnosis of type 1 diabetes ($p = 0.816$).

Gestational age was similar in type 1 diabetes patients and controls with a median (IQR) gestational age of 277 (267.5–281) days for type 1 diabetes patients and 276 (267–280) days for controls ($p = 0.48$), without any significant difference in the proportions of pre-term, term and post-term delivery ($p = 0.37$). There were also no statistically significant differences in mean birth weight of type 1 diabetes patients (3.28 ± 0.52 Kg) and controls (3.28 ± 0.56 Kg) ($p = 0.8$) or in the proportion of low birth weight, normal weight or foetal macrosomia ($p = 0.84$). Similarly, there was no statistically significant difference when birth weight was expressed as a percentage of 50th centile, which was calculated as the median birth weight for gestational age in grams (95.7% (88.2–108.0%) for type 1 diabetes patients; 100.6% (90.6–106.6%) for controls, $p = 0.41$) and when birth weight was calculated for gestational age to determine the number of children who were small for gestational age (SFGA), normal for gestational age (NFGA) or large for gestational age (LFGA) ($p = 0.47$).

Type 1 diabetes patients experienced a greater number of life events listed in the Holmes and Rahe Non-Adult Stress Scale together with a greater number of life events with a score of 50 or more life change units than controls. 25/89 type 1 diabetes patients experienced at least 1 life event compared to 14/89 controls ($p = 0.0014$) (Fig. 3) while 21/89 type 1 diabetes patients had a total stress score of 50 or more compared to 10/89 controls ($p = 0.0002$) (Fig. 4). The difference between the total stress score for type 1 diabetes and controls was also significant with a p value of <0.001 and a median (IQR) total stress score of 0 (0–62.5) for type 1 diabetes patients and 0 (0–0) for controls.

Smoking by the mothers and by the fathers before pregnancy, during pregnancy and during childhood up to

Table 1 Summary of results for type 1 diabetes subjects and controls

	Controls	Type 1 diabetes subjects	<i>p</i> value
Maternal age at delivery (years) ^a	29.1 ± 5.3	29.3 ± 4.6	0.63
Paternal age at delivery (years) ^a	31.5 ± 5.4	31.1 ± 5.0	0.25
Caesarian Section ^b	16 (18)	15 (16.9)	0.20
Birth Order ^c	1 (2–2)	1 (2–2)	0.96
Number of siblings in household until diagnosis ^c	1 (1–2)	0 (0–1)	0.82
Gestational Age (days) ^c	276 (267–280)	277 (267.5–281)	0.48
Birth weight (Kg) ^c	3.28 ± 0.56	3.28 ± 0.52	0.80
Birth weight as % of 50 th centile ^c	100.6 (90.6–106.6)	95.7 (88.2–108.0)	0.41
Maternal smoking before pregnancy ^b	15 (17.0)	22 (25.0)	0.20
Maternal smoking during pregnancy ^b	4 (4.5)	3 (3.4)	0.70
Maternal smoking during childhood ^b	15 (17.0)	16 (18.2)	0.81
Paternal smoking before pregnancy ^b	38 (45.2)	38 (45.2)	1.00
Paternal smoking during pregnancy ^b	35 (39.8)	38 (45.2)	0.65
Paternal smoking during childhood ^b	33 (37.5)	32 (36.4)	0.87
Maternal use of antibiotics during pregnancy ^b	9 (10.2)	10 (11.4)	0.81
Duration of exclusive breast feeding (days) ^c	2 (0–90)	1 (0–90)	0.2
Total duration of breast feeding (days) ^c	45 (0–150)	7 (0–90)	0.16
Age at introduction of formula milk (days) ^c	1 (1–91)	1 (1–31)	0.53
Age at introduction of cow’s milk (days) ^c	12 (12–18)	12 (12–12)	0.21
Age at introduction of solid foods (months) ^c	5 (4–6)	6 (4–6)	0.18
Cleaning of house ^c	10 (4–30)	10 (4–30)	0.10
Exposure to household pets ^b	46 (51.7)	43 (48.3)	0.55
Outdoor shoes removed indoors ^b	35 (39.8)	37 (42.0)	0.76
Allowed to get dirty ^b	43 (48.9)	32 (36.4)	0.09
Hand-washing prior to eating ^c	75 (75–100)	100 (75–100)	0.001
Frequency of bathing ^c	7 (7–7)	7 (7–10)	0.005
Age at first attendance to school or nursery (months) ^c	36 (36–36)	36 (36–36)	0.354
Total stress score ^c	0 (0–0)	0 (0–62.5)	<0.001

Significant results are shown in bold

^aData are mean ± standard deviation

^bData are number (percent)

^cData are median (interquartile range)

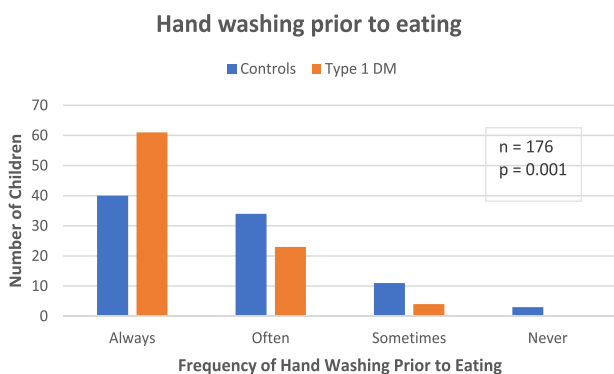


Fig. 1 Frequency of hand washing prior to eating for type 1 diabetes patients and controls. ‘*p*’ value refers to the statistical significance of the difference in the frequency distribution between subjects with type 1 diabetes and controls as assessed by the χ^2 test

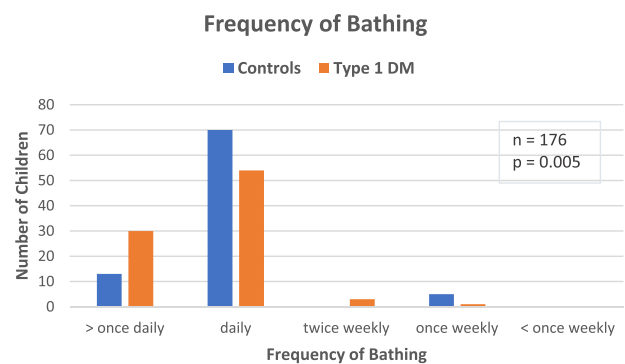


Fig. 2 Frequency of bathing for type 1 diabetes patients and controls. ‘*p*’ value refers to the statistical significance of the difference in the frequency distribution between subjects with type 1 diabetes and controls as assessed by the χ^2 test

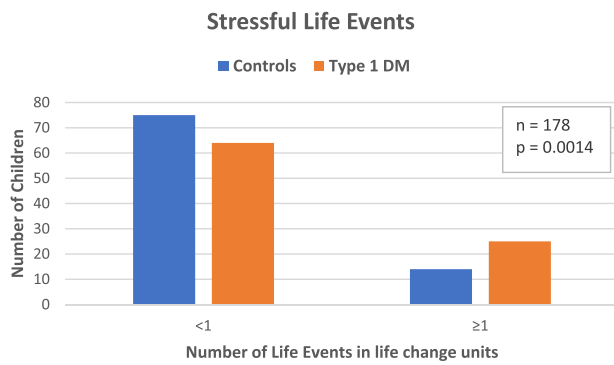


Fig. 3 Number of life events in the Holmes and Rahe Non-Adult Stress Scale for type 1 diabetes patients and controls until the age at diagnosis of type 1 diabetes. ‘*p*’ value refers to the statistical significance between subjects with type 1 diabetes and controls as assessed by the χ^2 test

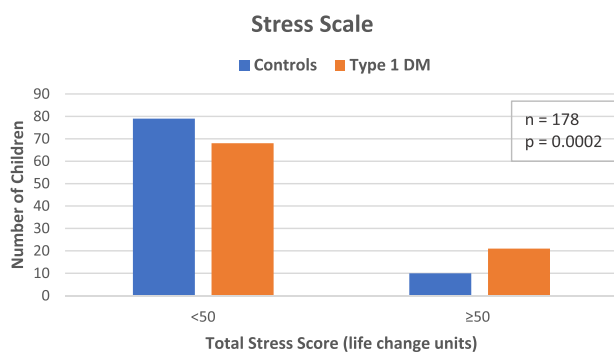


Fig. 4 Total stress score in life change units on the Holmes and Rahe Non-Adult Stress Scale for type 1 diabetes patients and controls until the age at diagnosis of type 1 diabetes. ‘*p*’ value refers to the statistical significance between subjects with type 1 diabetes and controls as assessed by the χ^2 test

diagnosis of type 1 diabetes were not found to be statistically different between type 1 diabetes patients and controls. 25% of mothers of type 1 diabetes patients smoked before pregnancy compared to 17% of mothers of controls ($p = 0.2$), while 3.4% of mothers of type 1 diabetes patients smoked during pregnancy compared to 4.5% of mothers of controls ($p = 0.7$). 18.2% of mothers of type 1 diabetes patients and 17.0% of mothers of controls smoked during childhood until the date of diagnosis of type 1 diabetes ($p = 0.81$). 45.2% of fathers of type 1 diabetes patients smoked during pregnancy compared to 39.8% of fathers of controls ($p = 0.65$). During childhood, 36.4% of fathers of type 1 diabetes patients smoked until the age of diagnosis of type 1 diabetes compared to 37.5% of fathers of controls ($p = 0.87$). 11.4% ($n = 10$) of mothers of type 1 diabetes patients used antibiotics during pregnancy compared to 10.2% of mothers of controls (9) ($p = 0.81$).

Type 1 diabetes patients and controls did not differ significantly in the total duration of exclusive breast feeding (median (IQR) of 1 day (0–90) for type 1 diabetes patients,

Table 2 Results of multiple logistic regression analysis as predictors of type 1 diabetes

	B	S.E.	Statistical significance	Odds ratio
Clean house	0.213	0.064	$p = 0.001$	1.24
Allowed to get dirty	−5.485	1.622	$p = 0.001$	0.004
Hand washing	0.031	0.009	$p = 0.001$	1.03
Bathing	−0.024	0.055	$p = 0.66$	0.98
Holmes and Rahe stress score	0.01	0.007	$p = 0.14$	1.01

median of 2 days (0–90) for controls; $p = 0.2$), exclusive breast feeding for 3 weeks or more ($p = 0.27$), 2 months or more ($p = 0.32$) and 3 months or more ($p = 0.55$), in the total duration of exclusive and non-exclusive breastfeeding (median (IQR) of 7 (0–90) days for type 1 diabetes patients, median of 45 days (0–150) for controls; $p = 0.16$) or in the duration of exclusive and non-exclusive breastfeeding for 4 months or more ($p = 0.53$). Age at introduction of formula milk and introduction of formula milk at 2 or more months was not significantly different between type 1 diabetes patients and controls. Median (IQR) age at the introduction of formula milk was 1 (1–31) days for type 1 diabetes patients and 1 (1–91) days for controls ($p = 0.53$). 23.6% of type 1 diabetes patients had formula milk introduced at 2 or more months compared to 33.7% of controls ($p = 0.17$). Similarly, no significant difference was observed in the age at introduction of cow’s milk with a median age of 12 months for both type 1 diabetes patients and controls (IQR 12–18 for type 1 diabetes patients; IQR of 12–12 for controls ($p = 0.21$). Age at introduction of cow’s milk was commonest at 12 to 17 months for both groups ($p = 0.94$). The median (IQR) age at introduction of solid food was 6 (4–6) months for type 1 diabetes patients and 5 (4–6) months for controls ($p = 0.178$).

Table 2 shows the results of multiple logistic regression analysis with type 1 diabetes being the dependent variable and parameters which were significant or quasi-significant ($p < 0.1$) in univariate analysis entered as co-variables. Frequency of cleaning the house (odds ratio, OR = 1.237, $p = 0.001$) and hand washing (OR = 1.031, $p = 0.001$) were independently associated with greater risk of type 1 diabetes, whilst being allowed to get dirty was associated with a lower risk (OR = 0.004, $p = 0.001$).

Discussion

Of all the prenatal and early life factors potentially associated with the development of type 1 diabetes included in this study, hand washing prior to eating, frequency of bathing and total stress score were the ones which were found to be positively associated with the development of

type 1 diabetes on univariate analysis with the first two being related to personal hygiene. Hand-washing prior to eating and frequency of house cleaning were independently associated with an increased risk of type 1 diabetes in multivariate analysis, whilst getting dirty was associated with a reduced risk. To date, there is very little data about these behaviours and type 1 diabetes risk in the literature. These findings are consistent with the hygiene hypothesis [14, 20] which suggests that lack of microbial exposure in early life predisposes to type 1 diabetes. It might also help explain the reported increase in incidence of type 1 diabetes [1, 2, 21] since the prevalence of infectious diseases has decreased and sanitation standards have improved over the same period. This also supports the ‘old friends hypothesis’, which suggests that the depletion of organisms from the urban environment that accompanied the evolution of mammals is one of the reasons for the increasing incidence of chronic inflammatory disorders since the mid-nineteenth century in developed countries [22]. Hand washing prior to eating, frequency of bathing and getting dirty affect the skin and gut microbiome. We did not find any association with the age of first attendance to school or pre-school which is a marker of respiratory infections in early life. The latter would be expected to affect the respiratory microbiome [23]. These results are consistent with lack of association between infections in early life and subsequent risk of type 1 diabetes reported from analysis of the General Practice Research Database practices in the UK [24], since most infections in this age group are likely to be respiratory. These data therefore suggest that factors which affect the skin or gut microbiome might be more important than infections or factors affecting the microbiome at other sites.

Sweat is source of ammonia [25], which can be oxidised by ammonia-oxidising bacteria (AOB) and ammonia-oxidising archaea (AOA) to nitrite and nitric oxide (NO) [26]. Archaea are prokaryotic cells which are distinct from bacteria. Frequent bathing may decrease these skin microbes. Getting dirty with soil may have the opposite effect since soil is a very good source of AOB and AOA [27, 28]. Frequent washing or bathing could therefore result in a reduction in the production of nitrite and NO possibly resulting in an adverse effect on T cell physiology, immunomodulation and immunoregulation. Nitrite and NO are rapidly and efficiently absorbed via the skin and nitrite can be converted to NO [29]. Skin AOB and AOA therefore constitute a biologically significant source of NO. High NO concentrations can shift the balance from a helper T-lymphocytes (Th) 1 to a Th2 response [30] and suppress Th17 [31]. Th1 cytokines promote a pro-inflammatory response which can predispose to autoimmune disease, whilst Th2 cytokines promote an IgE and eosinophilic responses responsible for atopy (reviewed by Romagnani [32]). Th17, on the other hand, stimulates cell types of both immune and non-immune nature to promote inflammation and

cell destruction [33]. The suppression of Th1 and Th17 by NO may protect against type 1 diabetes, whose pathogenesis is mainly mediated by Th1 and Th17 response [34]. Furthermore NO can suppress pancreatic β -cell apoptosis [35].

We also found hand washing prior to eating to be associated with an increased risk of type 1 diabetes in both univariate and multivariate analysis. The microbiome of skin of the hands is particularly diverse [36, 37]. Hand-washing prior to eating may therefore contribute to lack of diversity of the gut microbiome. It should be noted that the gut microbiome in type 1 diabetic subjects has been reported to exhibit less biodiversity with less butyrate-producing bacteria, resulting in suboptimal mucin synthesis [38]. Hand washing prior to eating probably reduces the exposure of the host to the ‘old friend’ microorganisms via the oral route, which are present in the natural environment. This can modify the composition of the gut microbiome, possibly also decreasing its bacterial biodiversity [39]. The microbiota of the natural environment can modulate the immune system either directly via direct colonisation of the human microbiota, indirectly via competition with or antagonism of established organisms [40], or via modification of the host-microbiota relationship following modulation of the immune system.

Similarly, the increased risk of type 1 diabetes associated with more frequent cleaning of the house on multivariate analysis in our study might also be related to decreased exposure of children to the immunoregulatory microorganisms from the natural environment in their homes. This may be mediated by a decrease in the biodiversity and/or altering the microbial communities found in the house through frequent cleaning of floors and surfaces and the frequent use of cleaning products. In a study by Dunn et al. [41], bacterial communities with lower levels of bacterial diversity within homes were typically found on surfaces that are regularly cleaned compared to infrequently cleaned surfaces. A large number of microorganisms are present in the air itself [42] which modulate the immune system once they reach the skin, the airways and the gut [39].

Risk of type 1 diabetes was also found to be positively associated with the total Holmes and Rahe stress score on univariate analysis in our study, which included the total significant life events which occurred prior to type 1 diabetes diagnosis for type 1 diabetes patients and the total significant life events which occurred up to the same age for controls. This is consistent with the findings of the All Babies in Southeast Sweden (ABIS) population-based prospective cohort study [43]. Stress modulates the immune system through various mechanisms. In our study, the association of the total Holmes and Rahe stress score with type 1 diabetes lost statistical significance in multivariate analysis; only hand washing prior to eating, cleaning of the house and being allowed to get dirty were found to be significant on

multivariate analysis. This suggests that the association of stress with type 1 diabetes risk may be mediated, at least in part, by the association of stress with increased hygiene. However, we cannot exclude a direct effect of stress on the immune system. Epinephrine stimulation of monocyte β -1 adrenergic receptors induces interleukin (IL)-1 β production [44]. Norepinephrine binds β -2 adrenergic receptors on antigen presenting cells facilitating IL-10 and IFN- γ production while downregulating TNF- α and IL-12 production [45]. During chronic stress, there is decreased monocyte sensitivity to glucocorticoids [46] and decreased capacity of glucocorticoids to suppress production of the pro-inflammatory cytokine IL-6. IL-1 β [47], IL-6 [48] and IL-10 [49] have all been linked to type 1 diabetes. However glucocorticoids, which are increased during stress, decrease monocyte production of IL-12 and tumour necrosis factor- α (TNF- α) [50]. Norepinephrine also downregulates TNF- α [45]. Since both IL-12 [49, 51] and TNF- α [52] have also been linked to type 1 diabetes, the effect of stress on type 1 diabetes risk might depend on the balance of these opposing effects, which in turn might depend on the influence of various genetic and environmental factors. Increased psychological stress may also lead to insulin resistance and increased demand on the pancreatic β -cell leading to endoplasmic reticulum strain and the formation of neoautoantigens and a breakdown in immune tolerance [53].

Our results did not confirm a protective effect of breast feeding, as been previously reported by various authors. This may be due to the very short duration of exclusive breast feeding in our cohort or due to small sample size. We also did not find an association with birth weight. Again this might be due to small size. It is important to note our aim was not study these factors, whose role is well-established, but to able to ensure that they do not act as potential confounders.

Strengths and limitations

A major strength of this study is that all the data of mothers of persons living with type 1 diabetes and mothers of controls was collected by the same interviewer thus maximising homogeneity of data collection. Individuals who were identified as controls were matched for sex and date of birth eliminating potentially confounding factors with respect to seasonality of birth.

Limitations of this study include small sample size and the retrospective rather than a prospective study, thus being subject to recall bias. Furthermore, genetic risk for type 1 diabetes in individuals included as controls in the study was not determined. We used a validated questionnaire to assess life-stress events, but not to assess childhood behavioural

habits as we could not find any which addresses the habits that we wished to investigate.

Conclusion

Our data show that hand-washing prior to eating and frequent house cleaning during childhood are independently associated with increased risk of type 1 diabetes, whilst getting dirty with soil is independently associated with reduced risk. These associations are likely to be mediated through alteration of the skin and gut microbiome.

Since maintaining good hand hygiene is of utmost importance in preventing the spread of potentially dangerous infections, the International Scientific Forum (IFH) (<http://www.ifh-homehygiene.org>) have put forward the recommendation of ‘targeted hygiene’. This highlights the importance of hygiene at specific points in the chain of possible transmission of infection, namely at points where adequate hygiene really matters to avoid spread of harmful microbes. This includes washing hands after using the toilet, handling of raw food, coughing, sneezing, nose blowing, handling and disposal of refuse, care of domestic animals and following contact with an infected family member. This would provide a balance between allowing adequate exposure to the old friend microorganisms which are necessary to maintain immunoregulation while concurrently preventing the spread of potentially dangerous infections. Other possible ways to reduce the risk of type 1 diabetes without increasing the risk of infections worth exploring is to alter the skin and gut microbiome by using soaps with ammonia-oxidising bacteria or by faecal transplantation. This requires further study.

In view of the current COVID-19 pandemic and the worldwide health promotion campaign regarding the importance of meticulous hand hygiene to prevent the spread of this viral disease, and with the subsequent widespread increased frequency and duration of hand washing, we need to study how these increased hygiene practices are going to impact the incidence of type 1 diabetes and other autoimmune diseases worldwide in the future.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by A.G.A. The first draft of the manuscript was written by S.F. and A.G.A. Both authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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