Pediatric Endocrinology Diabetes and Metabolism
Endokrynologia, Diabetologia i Choroby Przemiany Materii Wieku Rozwojowego

Praca oryginalna | Original Paper
Pediatr Endocrino Diabetes Metab 2014;21,3:95-100

The impact of having siblings – analysis of "hygiene theory" of chronic diseases in patients with type 1 diabetes in population of the Łódz region
Hygiene theory and type 1 diabetes

Wpływ posiadania rodzeństwa – analiza elementów „teorii higienicznej" chorób przewlekłych u chorych na cukrzycę typu 1 w populacji regionu łódzkiego
Teoria higieniczna i cukrzyca typu 1

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Abstract

Introduction. In the recent years there has been a significant increase in the incidence of the type 1 diabetes mellitus. Therefore, numerous studies are underway to evaluate the possible factors underlying this trend. Some studies suggest that better sanitary conditions and lack of contact with microorganisms might be important, thus increasing the risk of disease in firstborns. Moreover, siblings could play an important role in the transmission of pathogens, which, by stimulating the immune system, may prevent the development of atopic and autoimmune diseases including such as type 1 diabetes. Current data, however, are still inconclusive. **Purpose.** The aim of the study was to evaluate the effect of having siblings on the incidence of type 1 diabetes among children and adults. **Materials and methods.** A group of 469 patients with type 1 diabetes was selected. The study population was composed of 245 adults and 224 youth patients. Information from Outpatient Diabetologic Departments database was gathered. Data such as age at the diagnosis of diabetes, sex of siblings, number and birth order were analyzed. **Results.** In the studied population, 4.5% were only children, and 30.3% patients came from large families. In the group of type 1 diabetic patients 39.7% were firstborns and this proportion was comparable to the group of healthy subject. The highest proportion of firstborns was noted in the group that was diagnosed after 18 years of age (45,1%) compared to the group that was diagnosed between 10 and 14 (29,1%) ($p<0.05$). Type 1 diabetic patients that were not firstborns much more often had older siblings of the opposite sex than the same sex. **Conclusions.** he firstborns in the population of type 1 diabetes from the Łódz region did not outnumber the healthy subjects. Significantly higher proportion of firstborns in the group that was diagnosed after 18 years of age compared to the group that was diagnosed between 10 an 14 years was noted.

Key words
diabetes mellitus type 1, hygiene hypothesis, siblings

Streszczenie

Wprowadzenie. W ostatnich latach nastąpił znaczny wzrost zachorowalności na cukrzycę typu 1. W związku z tym trwają liczne badania oceniające prawdopodobne przyczyny tego trendu. Niektóre z nich sugerują, że lepsze warunki higieniczne i brak kontaktu z mikroorganizmami mogą mieć znaczenie w tym zakresie, zwiększając ryzyko zachorowania u osób pierworodnych. Ponadto rodzeństwo może odgrywać istotną rolę w przekazywaniu patogenów, które poprzez stymulację układu immunologicznego mogą zapobiegać rozwojowi chorób atopowych i autoimmunologicznych, w tym takich jak np. cukrzyca typu 1. **Cel pracy.** Celem pracy była ocena wpływu posiadania rodzeństwa na zachorowalność na cukrzycę typu 1 wśród dzieci i dorosłych. **Materiał i metody.** Do badania włączono grupę 469 pacjentów z DM1, w tym 245 dorosłych oraz 224 dzieci. Dane zgromadzono na podstawie informacji zawartych w bazach danych Poradni Diabetologicznych. Analizie poddano wiek zachorowania na cukrzycę, płeć posiadanego

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Received: 2014-04-12.
Acccepted: 2014-10-10.
Conflict of interests: none declared.

rodzeństwa, liczbę oraz kolejność urodzenia. **Wyniki.** W analizowanej populacji 4,5% stanowili jedynacy, a 30,3% pacjentów pochodziło z rodzin wielodzietnych. W ocenianej grupie chorych na cukrzycę osoby pierworodne stanowiły 39,7% i odsetek ten był porównywalny do grupy osób zdrowych. Największy odsetek osób pierworodnych znalazł się w grupie pacjentów, którzy zachorowali w wieku powyżej 18 roku życia (45,1%), najmniejszy zaś w grupie pomiędzy 10 a 14 rokiem życia (29,1%) ($p<0.05$). Chorzy na cukrzycę typu 1 niebędący osobami pierworodnymi częściej posiadali starsze rodzeństwo o płci odmiennej. **Wnioski.** W grupie osób chorujących na cukrzycę typu 1 z obszaru województwa łódzkiego nie zaobserwowano przewagi liczebnej osób pierworodnych w porównaniu do grupy osób zdrowych. Zanotowano istotnie wyższy odsetek osób pierworodnych w grupie, która zachorowała po 18 roku życia w porównaniu do grupy, która zachorowała pomiędzy 10 a 14 rokiem życia.

Słowa kluczowe
cukrzyca typu 1, teoria higieniczna, rodzeństwo

Introduction

Type 1 diabetes is a chronic autoimmune disease characterized by pancreatic β cell destruction. However, the etiology of this disease has not been clearly discovered so far. In recent years there has been a significant increase in the incidence of type 1 diabetes mellitus (1,2,3). The results of the international program EURODIAB demonstrate that between the year 2005 and 2020 the number of new cases of type 1 diabetes in children under 5 years of age will double if the current increasing trend maintains. Furthermore, the total number of new cases in subjects under 14 years of age will increase by 70% (2). Therefore, there is a need for further research in this field and all the possible causes of this trend are under evaluation.

It is postulated that one of the reasons for this trend may be due to limited contact with microorganisms during the maturation of the immune system (so-called hygiene theory of chronic diseases). In 1989, David Strachan suggested that environmental factors, such as economic status, parents' education and number of siblings might have an impact on the frequency of allergic urticaria incidence (4). These observations were crucial for further development of the hygiene theory. Its assumptions are based on the suggested association between better sanitary conditions and the prevalence of allergic and autoimmune diseases. On the molecular background, it is explained by the balance shift between of Th1 and Th2 lymphocyte populations, with a predominance of the latter (5). The hygiene hypothesis postulates the impact of many different factors, including, among others, both maternal and paternal age at birth, birth weight, incidence of mother's gestational diabetes, cigarette smoking by the parents and socioeconomic status of the family. An important role in this theory is attributed to the siblings. They are supposed to play a vital role in the transmission of various pathogens, such as viruses, fungi, bacteria, and parasites, which is essential for the proper development and maturation of the immune system. Upbringing in the household with the siblings enables the organism to learn the proper response to antigens to which the body is exposed. According to the hygiene theory, not only the number of siblings but also the birth order matters. It is believed that the only children and first-borns are usually provided with better hygienic care, due to more meticulous and often excessive hygiene practices. Moreover, the child who older siblings is also exposed to more antigens during pregnancy as they are transmitted from older siblings to the mother. Data on the incidence of type 1 diabetes in this aspect are limited, but they seem to confirm the basic assumptions of the theory (6,7).

Due to the observed increasing trend in the incidence of type 1 diabetes, together with a simultaneous improvement of hygienic conditions and the noted decline in fertility rate, the hygiene hypothesis seems of interest in terms of possible preventive or therapeutic strategies. Analysis of The World Factbook indicates that the total fertility rate in Poland (number of births per woman) is among the lowest in the world (212th place of 224 countries) and is as high as 1.33. Moreover, it decreased from 2.1 in 1990 to 1.3 in 2011, so it decreased for about 40% over the period of 20 years. Therefore, we decided to investigate the element of the hygiene theory concerning the role of siblings among the patients with type 1 diabetes in the Łódz region.

Aim of the study

The aim of the study was to evaluate the effect of having siblings on the incidence of type 1 diabetes among children and adults.

Materials and Methods

Retrospective analysis included a group of 469 patients with type 1 diabetes, including 245 adults aged 19 to 65 years (median 19 years) and 224 children between the age of 2 to 18 years (median 7 years). The patients were treated in the Outpatient Department at the Polish Mother's Memorial Hospital/Research Institute and the Pediatric Outpatient Department of the University Teaching Hospital No 4 in Łódz and the data were gathered between September 2013 and January 2014. Total number of 1,127 subjects, including type 1 diabetes patients together with their siblings, was finally analyzed. All the data were retrieved from the records of outpatient departments database. Only those patients, whose files included complete information about their siblings, were included in the study. Information concerning the patients such as sex, age, and age at diagnosis of diabetes was recorded. The data regarding the siblings included the number of siblings, sex, birth order and age at diagnosis of type 1 diabetes in the siblings if applicable.

All the data were then statistically analyzed with $p<0.05$ considered as statistically significant. Fisher's exact test was used to compare the proportions of subjects between the groups.

Results

In the studied population, majority of families had two children, less than a third of patients came from large families (with 3 or more children) while 25 people were the only child (figure 1).

Among 9 patients with twin siblings (4 identical and 5 fraternal twins) in 5 cases diabetes was diagnosed in both twins (in 2 identical and 3 fraternal twins). Families in which more than one child suffered from type 1 diabetes accounted for 4.3% of the population (figure 2).

In the entire cohort of type 1 diabetic patients 39.7% were first-borns while in a population of healthy siblings the group of first-borns accounted for 44%. The difference in the proportion of the first-borns between the two groups was no significant (figure 3).

The mean age at the diagnosis of diabetes was 11 years. The highest proportion of the first-borns was noted in the group of patients who were diagnosed with diabetes after 18 years of age (45.1%), while the smallest percentage was observed in the group with diabetes onset between 10 and 14 years of age (29.1%). The difference between those two groups was statistically significant ($p<0.05$). There were no significant differences in the proportion of first-borns between the remaining groups (figure 4).

In the group of type 1 diabetic patients that weren't first-borns, the older siblings were much more often of the opposite sex than the same sex ($p<0.05$) (figure 5).

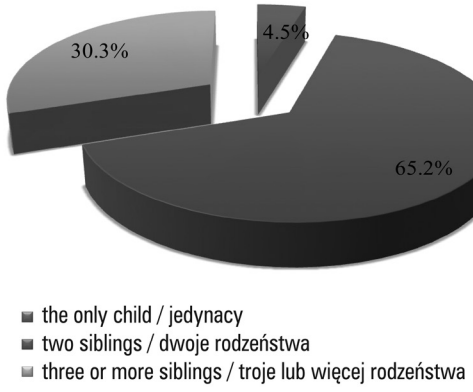

Fig 1. Number of siblings in families
Ryc 1. *Liczba rodzeństwa w rodzinach*

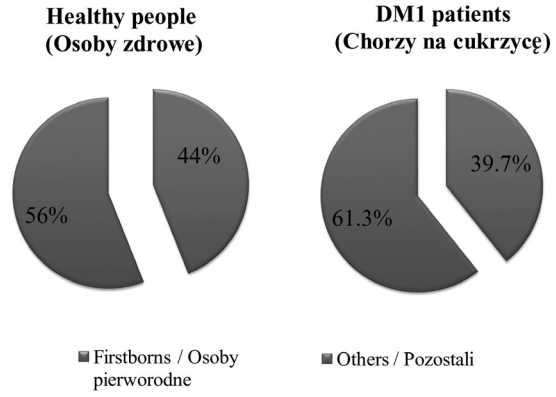

Fig. 3. Number of firstborns
Ryc. 3. *Liczba osób pierworodnych*

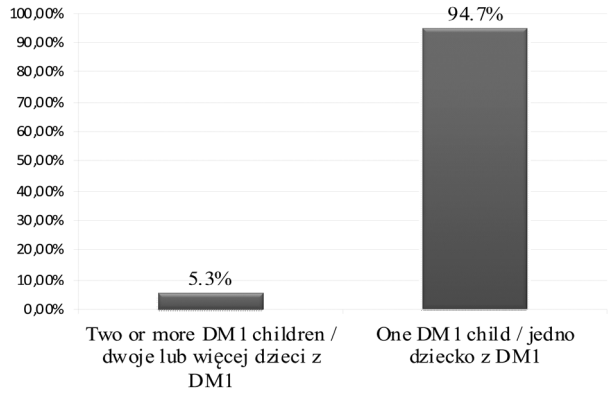

Fig. 2. Number of diabetic children in families
Ryc. 2. *Liczba dzieci chorych na cukrzycę w rodzinach*

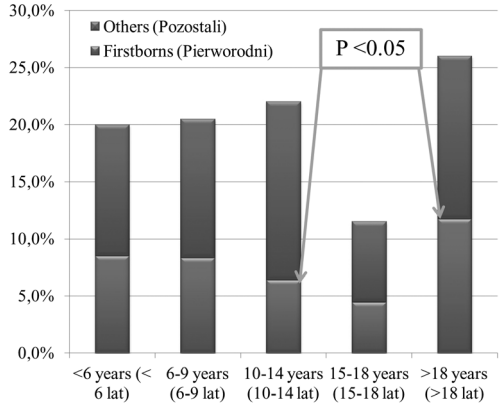

Fig. 4. Age at onset of diabetes
Ryc. 4. *Wiek w momencie rozpoznania cukrzycy*

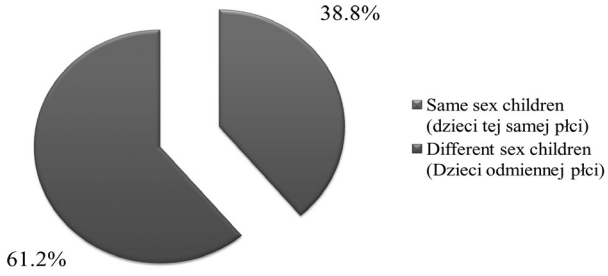

Fig. 5. Sex distribution in younger siblings with diabetes
Ryc. 5. *Rozkład płci u młodszego rodzeństwa chorującego na cukrzycę*

Discussion

The incidence of type 1 diabetes mellitus is constantly growing worldwide (1-3,8). It has been reported that its global prevalence is rising by 3% per year (3). Therefore, there is a constant debate on the potential theories explaining this trend and all the triggering factors are being explored. Pathogenesis of type 1 diabetes mellitus is complex and not yet fully elucidated. It is widely known that both genetic and environmental factors play a role. The risk associated with genetic factors is accounted for about 60-70% and is generally assumed to be rather stable. Therefore, the noted alarming increase in the incidence rate of type 1 diabetes is supposed to be due to environmental factors. There are many hypotheses explaining pathogenesis of type 1 diabetes that can be found in the literature: viral theory, hypothesis associated with vitamin D deficiency, cow's/breast milk theory, and finally hygiene theory. Current evidence concerning every single theory is conflicting and it seems that its pathogenesis should be explained as a multifactorial process and a complex interaction of many factors with a balance shift toward the development of type 1 diabetes.

The hygiene theory is thought as one of the most credited in pathogenesis of type 1 diabetes. It suggests that improved hygiene and living conditions, both at the national and individual level, prevent childhood infections to some extent and, as a result, attenuate the proper development of the immune system, thus increasing the risk of autoimmune and allergic diseases. Tamayo et al. in the update on Diabetes in Europe points to the hygiene theory as one of the pathogenic factors of type 1 diabetes (9). The first observation concerning the above mentioned association was published in 1989 by David Strachan, who noted for the first time that exposure to infectious agents during early childhood may play a protective role as concerns the incidence of allergy (4). The risk of hay fever in his study was inversely related to the number of siblings and this association was stronger in the older than younger siblings. It has been demonstrated that the incidence of many infectious diseases such as tuberculosis, enterovirus infections, measles or mumps is decreasing nowadays (10,11). In contrast, a global rise in the prevalence rate of type 1 diabetes has been documented. Available data from both animal and human studies seem to suggest that this observation is rather a causal relationship that just a random coincidence.

It has been observed that the incidence rate of type 1 diabetes in non-obese diabetic mice was higher when bred together with pathogen-free environment-bred animal. Moreover, the prevalence additionally increased by up to 80% when they were bred in isolation (11). Many experiments have demonstrated that infection with certain pathogens may prevent the development of type 1 diabetes (12). There are several potential immunological mechanisms that are supposed to underlie the hygiene hypothesis. Infectious epitopes seem to prevent autoreactive T-cells hyperactivity by interacting with many different receptors, finally leading to maintenance of balance between T-helper 1 cells, involved with immune response to infections, and T-helper 2 cells associated with the development of atopy. Limited exposure to infectious agents early in life, resulting from improved sanitary conditions and sterile food, favors up-regulation of autoreactive Th2 lymphocytes (12).

Having the siblings seems to be protective as they play an important role in the transmission of viral, bacterial and fungal infections, as well as parasites. Many studies have demonstrated an inverse association between an increasing number of siblings and the risk of type 1 diabetes (6,7). Birth order seems also important as it is assumed that the firstborns, during earliest childhood, grow in much more hygienic conditions than the following children (13). In our observation, we did not observe any difference in the firstborns proportion between the patients and their healthy siblings. The number of children in the families in our study was comparable to the mean distribution of children in the analyzed Łódz area (Central Statistical Office of Poland – Information Portal data). The vast majority of studies points that the prevalence rate of type 1 diabetes decreases together with an increase in the number of siblings (6). Strachan et al. have noted the reduced incidence rate of hay fever in the subjects of younger mothers and those from larger families but the number of older siblings was much more important than the number of younger siblings (14). However, there were also some studies that did not fully confirm such association. Svanes et al., in a study performed on the group of 13932 subjects from 36 areas in Europe, New Zealand, the United States, and Australia, have observed that the incidence of atopy was inversely associated with family size that partly resulted from an independent protective effect of a greater number of brothers. However, analyzing the total number of siblings, no further association was noticed concerning the number of siblings (15). Similarly, the study of Strachan et al. has revealed that the prevalence of allergy decreased with increasing number of brothers and was unrelated to the number of sisters (16). In our study we have found that type 1 diabetes subjects that were not firstborns much more often had older siblings of the opposite sex than the same sex, suggesting that sex distribution within the siblings is also of importance. This could be explained by the fact that the siblings of the same sex much more often play together, share the same toys, rooms or beds, so the interaction and potential microorganism transmission is much more likely. Marshal et al. have noted a lower type

1 diabetes risk in the children that shared the room with the sibling or had the social contacts with other children in early childhood (17). Svanes et al. have observed that bedroom sharing was related to a lower incidence of atopy. The beneficial effect of sharing a bedroom in this study, similarly like the impact of family size, could be noticed only in patients with no parental allergy, suggesting that in the subjects with a strong genetic predisposition, environmental factors seem of less importance and serve as additional modulating factors only (15).

There are many other environmental factors potentially related to the"hygiene theory of the allergic/autoimmune diseases", among others, parental education (17), maternal smoking during pregnancy as well as maternal infections and other illnesses in pregnancy (17), maternal age (17), socioeconomic factors that determine medical care access (7), attending day care (18) or breastfeeding (19). Cardwell et al. have observed in the area of Northern Ireland that increased type 1 diabetes risk was associated with higher maternal age, paternal age, birth weight and lower gestational age. Inverse association with birth order was noted but this relationship was apparent only after adjustment for maternal age and, what is more, it was present only for diabetes diagnosed under the age of five years (13). D'Angeli et al. have assessed the relationship between many maternal and infant factors and the risk of type 1 diabetes in 1852 type 1 diabetic patients, compared to 7408 control subjects. The results have shown that type 1 diabetes was negatively associated with having older siblings as well as with indicators of lower economic status or care access, among them: an unmarried mother, inadequate prenatal care or medical insurance (6). There are some other studies that connect socioeconomic status with type 1 diabetes incidence. Patterson et al. have noticed that poorer areas, with high population density, had the lowest incidence rate of type 1 diabetes, with household crowding as an important protective factor (20). Relationship has been observed between higher gross domestic product as well as lower infant mortality and higher incidence of type 1 diabetes (21). Increasing prevalence rate of type 1 diabetes in rapidly developing countries was also noted (22). Exploring the habitants of a single country, a higher prevalence rate was noted in the groups with higher socioeconomic status (23). Moreover, increased incidence of type 1 diabetes was demonstrated in populations migrating from low-incidence to high-incidence areas (24). Collective hygiene in developed countries concerns the factors such as the quality of drinking water and food, vaccination, excessive use of antibiotics and disinfectants. It has been demonstrated that large-scale medical interventions aimed at eliminating some pathogens (*Streptococcus pneumonie* in South Africa, helminth in Venezuela and Gabon) resulted in the rise in the incidence of atopy (25). There is also some evidence that not only overt infections but also some non-viable microbial compounds may play a role in this theory. It has been observed in many studies that children growing up on the farm have much lower incidence rate of allergic diseases (16). It is widely known that these children are exposed to many microbial antigens such as animal sheds, hay lofts, consumption of unpasteurized cow's milk and many farm activities that seem to exert a protective effect (26). It could be speculated that this effect was partially due to the fact that families living on the farms are bigger on average (16). Many observations revealed the decreased risk of type 1 diabetes in more crowded living conditions (20, 27). However, in the cited paper of Strachan higher number of siblings and exposure to farming compounds exerted independent protective effect on the risk of allergy (16). Svanes et al. have observed that the presence of a dog in the house during childhood was inversely associated with atopy in adulthood and this effect remained significant after adjustment for parental allergy, sibling allergy as well as adult pet ownership (15). Other studies also revealed that regular contact with animals was protective (17). The time of exposure also seems of importance as early exposure to environmental antigens has a much stronger effect than the same contact but after the first year of life (26). However, other studies do not reveal any significant association between infectious illnesses in infancy and the risk for hay fever (14). It was also observed that maternal contact with animal sheds during pregnancy protected the offspring from allergy (26). A north to south gradient in the incidence of type 1 diabetes has been observed, with much higher prevalence in the northern countries. However, increasing incidence is observed nowadays in southern countries (28). It is also worth noting that the prevalence rate of type 1 diabetes varies significantly not only between but also within the countries (29). It is thought that environmental rather than genetic factors underlie these variations. Staines et al. have observed significant differences in the incidence of type 1 diabetes between three regions of the northern England, noting that it was significantly lower in regions of higher population density and overcrowded houses (16). However, there were also some studies in which no differences in the incidence of type 1 diabetes within countries were observed (30).

The results concerning the role of infections presented in current literature are conflicting as another leading theory associated with type 1 diabetes pathogenesis is a viral theory, according to which certain viral infections increase the risk of the disease. Therefore, a body of evidence coexists indicating their both protective and harmful role as concerns autoimmune/atopic diseases. It is suggested that the observed harmful effect of certain pathogens result from antigen mimicry or triggering local inflammatory processes thus increasing immunogenicity of autoantigens. It cannot be excluded that the final influence of infecting pathogens depends on some factors not yet clearly elucidated. It is suggested that the time of exposure, the intensity of infection, the condition of immunological system and other factors may be of relevance. It is known, for example, that lower respiratory tract infections are known to be a risk factor for persistent wheeze and asthma, while the contact with pathogens when transmitted by other children (siblings in the household or other children during day care attendance) seems protective. Therefore, an important question appears – what should we do with undesirable and potentially harmful consequences of improved hygiene? It is undoubted that elimination of certain types of infections is one of the most important achievements in medicine. It seems, therefore, that a golden balan-

ce might be achieved via novel preventive and/or therapeutic approaches that would substitute these infections.

There are several limitations of our study that must be addressed. First of all we did not explore the whole population of the Łódz region. Secondly, relatively low number of the only children was taken into account. Additionally, we did not assess any other potentially confounding components of the hygiene theory, so it must be stressed that our conclusions concern only those factors of the theory that have been taken into consideration.

Conclusions

In the patients suffering from type 1 diabetes from the Łódz region, no superiority of the firstborns was observed compared to the healthy subjects. Significantly higher proportion of firstborns in the group that was diagnosed after 18 years of age compared to the group that was diagnosed between 10 an 14 years was noted. Therefore, the theory of the hygiene etiology of chronic diseases in this population was not confirmed. On the basis of the presented literature it seems that there is still a need for further research in this field in order to search for possible causes of the alarming upward trend in the incidence of type 1 diabetes.

Wyniki pracy zostały zaprezentowane w formie plakatu na zjeździe Polskiego Towarzystwa Diabetologicznego, Gdańsk 2014.

References

1. Jarosz-Chobot P, Polanska J, Szadkowska A, et al. Rapid increase in the incidence of type 1 diabetes in Polish children from 1989 to 2004, and predictions for 2010 to 2025. Diabetologia. 2011;54(3):508-15.
2. Patterson CC, Dahlquist GG, Gyürüs E, et al. Incidence trends for childhood type 1 diabetes in Europe during 1989—2003 and predicted new cases 2005—20: a multicenter prospective registration study. Lancet 2009;373(9680):2027-33.
3. Onkamo P, Väänänen S, Karvonen M, Tuomilehto J. Worldwide increase in incidence of Type I diabetes--the analysis of the data on published incidence trends. Diabetologia. 1999;42(12):1395-403. Erratum in: Diabetologia 2000;43(5):685.
4. Strachan DP. *Hay fever, hygiene, and household size.* BMJ. 1989;299(6710):1259-60.
5. Folkerts G, Walzl G, Openshaw PJ. *Do common childhood infections 'teach' the immune system not to be allergic?* Immunol Today. 2000;21(3):118-20.
6. D'Angeli MA, Merzon E, Valbuena LF, et al. *Environmental factors associated with childhood-onset type 1 diabetes mellitus: an exploration of the hygiene and overload hypotheses.* Arch Pediatr Adolesc Med. 2010;164(8):732-8.
7. Vlajinac H, Sipetić S, Marinković J, et al. *The Belgrade childhood diabetes study – comparison of children with type 1 diabetes with their siblings*. Paediatr Perinat Epidemiol. 2006;20(3):238-43.
8. Gale EA. *The rise of childhood type 1 diabetes in the 20th century*. Diabetes. 2002;51(12):3353-61.
9. Tamayo T, Rosenbauer J, Wild SH, et al. *Diabetes in Europe: an update.* Diabetes Res Clin Pract. 2014;103(2):206-17.
10. Tracy S, Drescher KM, Jackson JD, et al. *Enteroviruses, type 1 diabetes and hygiene: a complex relationship.* Rev Med Virol. 2010;20(2):106-16.
11. Bach JF. *The effect of infections on susceptibility to autoimmune and allergic diseases.* N Engl J Med. 2002;347(12):911-20.
12. Egro FM. *Why is type 1 diabetes increasing?* J Mol Endocrinol. 2013;51(1):R1-13.
13. Cardwell CR, Carson DJ, Patterson CC. *Parental age at delivery, birth order, birth weight and gestational age are associated with the risk of childhood Type 1 diabetes: a UK regional retrospective cohort study*. Diabet Med. 2005;22(2):200-6.
14. Strachan DP, Taylor EM, Carpenter RG. *Family structure, neonatal infection, and hay fever in adolescence*. Arch Dis Child. 1996;74(5):422-6.
15. Svanes C, Jarvis D, Chinn S, Burney P. *Childhood environment and adult atopy: results from the European Community Respiratory Health Survey.* J Allergy Clin Immunol. 1999;103(3 Pt 1):415-20.
16. Strachan DP, Harkins LS, Golding J. *Sibship size and self-reported inhalant allergy among adult women. ALSPAC Study Team.* Clin Exp Allergy. 1997;27(2):151-5.
17. Marshall AL, Chetwynd A, Morris JA, et al. *Type 1 diabetes mellitus in childhood: a matched case control study in Lancashire and Cumbria, UK*. Diabet Med. 2004;21(9):1035-40.
18. McKinney PA, Okasha M, Parslow RC, et al. *Early social mixing and childhood Type 1 diabetes mellitus: a case-control study in Yorkshire, UK*. Diabet Med. 2000;17(3):236-42.
19. Verge CF, Howard NJ, Irwig L, et al. *Environmental factors in childhood IDDM. A population-based, case-control study*. Diabetes Care. 1994;17(12):1381-9.
20. Patterson CC, Carson DJ, Hadden DR. *Epidemiology of childhood IDDM in Northern Ireland 1989-1994: low incidence in areas with highest population density and most household crowding. Northern Ireland Diabetes Study Group*. Diabetologia. 1996;39(9):1063-9.
21. Patterson CC, Dahlquist G, Soltész G, Green A; EURODIAB ACE Study Group. Europe and Diabetes. *Is childhood-onset type I diabetes a wealth-related disease? An ecological analysis of European incidence rates*. Diabetologia. 2001;44 Suppl 3:B9-16.
22. Stipancic G, La Grasta Sabolic L, Malenica M, et al. *Incidence and trends of childhood Type 1 diabetes in Croatia from 1995 to 2003.* Diabetes Res Clin Pract. 2008;80(1):122-7.
23. Haynes A, Bulsara MK, Bower C, et al. *Independent effects of socioeconomic status and place of residence on the incidence of*

childhood type 1 diabetes in Western Australia. Pediatr Diabetes. 2006;7(2):94-100.
24. Bodansky HJ, Staines A, Stephenson C, et al. *Evidence for an environmental effect in the aetiology of insulin dependent diabetes in a transmigratory population.* BMJ. 1992;304(6833):1020-2.
25. Bach JF. *Six questions about the hygiene hypothesis.* Cell Immunol. 2005;233(2):158-61. Review.
26. Riedler J, Braun-Fahrländer C, Eder W, et al. *Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey.* Lancet. 2001;358(9288):1129-33.
27. Staines A, Bodansky HJ, McKinney PA, et al. *Small area variation in the incidence of childhood insulin-dependent diabetes mellitus in Yorkshire, UK: links with overcrowding and population density.* Int J Epidemiol. 1997;26(6):1307-13.
28. Mazzella M, Cotellessa M, Bonassi S, et al. *Incidence of type I diabetes in the Liguria Region, Italy. Results of a prospective study in a 0- to 14-year age-group.* Diabetes Care. 1994;17(10):1193-6.
29. Waugh NR. *Insulin-dependent diabetes in a Scottish region: incidence and urban/rural differences.* J Epidemiol Community Health. 1986;40(3):240-3.
30. Levy-Marchal C, Papoz L, de Beaufort C et al. *Incidence of juvenile type 1 (insulin-dependent) diabetes mellitus in France.* Diabetologia. 1990;33(8):465-9.