OBJECTIVE — Type 1 diabetes is associated with a wide spectrum of susceptibility and protective genotypes within the HLA class II system. It has been reported that adults diagnosed with youth-onset type 1 diabetes more recently have been found to have fewer classical high-risk HLA class II genotypes than those diagnosed several decades ago. We hypothesized that such temporal trends in the distribution of HLA-DR, DQ genotypes would be evident, and perhaps even stronger, among 5- to 17-year-old Hispanic and non-Hispanic white (NHW) youth diagnosed with type 1 diabetes in Colorado between 1978 and 2004.

RESEARCH DESIGN AND METHODS — HLA-DR, DQ was typed using PCR and sequence-specific oligonucleotide hybridization in 100 youth diagnosed during the period of 1978–1988 and 264 diagnosed during 2002–2004. Logistic regression was used to adjust for confounders and assess temporal trends.

RESULTS — The frequency of the highest-risk genotype (DRB1*03-DQB1*02/DRB1*04-DQB1*03) was higher (39%) in children diagnosed during the period 1978–1988 than in those diagnosed during 2002–2004 (28%). A similar pattern was observed in NHWs and Hispanics.

CONCLUSIONS — We found that high-risk HLA genotypes are becoming less frequent over time in youth with type 1 diabetes of NHW and Hispanic origin. This temporal trend may suggest that increasing environmental exposure is now able to trigger type 1 diabetes in subjects who are less genetically susceptible.
from 1 January 1978 to 31 December 1988 (10,11). SEARCH is a multicenter, population-based observational registry that ascertained new cases of physician-diagnosed diabetes in youth 0–19 years of age from 1 January 2002 forward (12). A detailed description of this population (2), the Colorado Type 1 Diabetes Registry (10,11), and SEARCH have been published (12). Type 1 diabetes was defined as use of insulin within 2 weeks from diagnosis. Completeness of case ascertainment was assessed by the capture-recapture method (13,14) and estimated to be 96–97% over time (2).

Youth aged 5–17 years identified with incident type 1 diabetes during the period of 1978–1988 and 2002–2004 were eligible to participate in this analysis. In 1978–1988, all Hispanic youth (n = 120) and a similar size random sample of NHW (n = 122) youth identified by the Colorado Type 1 Diabetes Registry were eligible for genetic typing, and 41 Hispanic (34%) and 59 NHW youth (48%) had HLA measured (15). In 2002–2004, all youth over the age of 4 years of Hispanic (n = 87) or NHW (n = 512) origin were eligible for genetic typing. A total of 39 Hispanic (45%) and 225 NHW (44%) youth participated. Age at diagnosis, sex, and family history of diabetes were assessed to determine whether the youth with HLA measured were representative of the overall registered population in each period and racial/ethnic group.

Date of diagnosis and date of birth were obtained from medical records. Family history of diabetes was obtained from medical record abstraction (71% in 1978–1988 and 85% in 2002–2004) or from self-administered questionnaires completed within 5 years from the date of diagnosis (17% in 1978–1988 and 3% in 2002–2004). Children who had a sibling, parent, or grandparent with any type of diabetes were defined as having a positive family history.

To assess whether the sample of youth with available HLA typing was representative of the entire patient population of youth with type 1 diabetes diagnosed in these two time periods, demographic and clinical characteristics of study participants were compared with those of nonparticipants. Table 1 shows these characteristics according to time period and ethnicity. In 1978–1988, there were no significant differences for either NHW or Hispanic youth. In 2002–2004, the only significant difference between those with and without HLA typing was a higher frequency of a positive family history of diabetes in NHW participants with typing available. However, no significant relationship was noted between having the highest-risk HLA genotype (DRB1*03-DQB1*02/DRB1*04-DQB1*03) and having a positive family history of diabetes in either time period (P = 0.96). These data suggest that our samples of NHW and Hispanic youth with measured HLA are likely representative of the larger eligible population with type 1 diabetes in both periods, or that they differ in characteristics not strongly related with the frequency of high-risk HLA genotypes. Therefore, we believe that any observed trends or lack thereof in the frequency and distribution of HLA genotypes over time in our population are not likely to be due to selection bias. All participants provided informed consent and both the Colorado Type 1 Diabetes Registry and SEARCH were approved by relevant institutional review boards.

## HLA typing

DRB1 and DQB1 typing was performed using One Lambda PCR assays that were standard for the time period in which they were used (1978–1988 and 2002–2004) (16). The main difference between assays was the number of DQB1 alleles identified that had sequence-specific oligonucleotides (SSOs) available for typing. There were seven DQB1 SSOs identified for typing in 1978–1988, and there were 100 DQB1 SSOs identified for typing in 2002–2004. Due to the difference in the number of DQB1 alleles identified and the availability of SSOs for typing these alleles over the two periods, only the gene locus was used in the analysis to compare genotypes (e.g., DQB1*02 vs. DQB1*0201). Additionally, the current PCR sequence-specific oligonucleotide probes used in 2002–2004 allowed for a larger number

### Table 1—Characteristics of the study population by time period (1978–1988 and 2002–2004) and participation status in the HLA typing

| Characteristic               | NHW Nonparticipants | NHW Participants | P     | Hispanic Nonparticipants | Hispanic Participants | P     |
|-----------------------------|----------------------|------------------|-------|--------------------------|-----------------------|-------|
| 1978–1988                   | 58                   | 59               | 122   | 75                       | 41                    | 120   |
| n                           | 54/46                | 45/55            | 0.17  | 37/63                    | 41/59                 | 0.66  |
| Family history (%yes/no)    | 54/46                | 44/56            | 0.15  | 55/45                    | 69/31                 | 0.16  |
| Age-group at diagnosis (years) | 38 (22)             | 41 (24)          |       | 28 (21)                  | 32 (13)               |       |
| 5–9                         |                      |                  |       |                          |                       |       |
| 10–14                       | 48 (28)              | 42 (25)          | 0.64  | 52 (39)                  | 57 (23)               | 0.42  |
| 15–17                       | 14 (8)               | 17 (10)          |       | 20 (15)                  | 11 (5)                |       |
| Mean age at diagnosis (years) | 11.2 ± 3.3          | 11.1 ± 3.4       | 0.73  | 11.8 ± 3.1               | 11.2 ± 3.0            | 0.27  |
| 2002–2004                   | 287                  | 225              | 512   | 48                       | 39                    | 87    |
| n                           | 53/47                | 53/47            | 0.95  | 56/44                    | 49/51                 | 0.48  |
| Family history (%yes/no)    | 48/52                | 58/42            | 0.04  | 59/41                    | 69/31                 | 0.32  |
| Age-group at diagnosis (years) | 38 (109)            | 38 (86)          |       | 40 (19)                  | 36 (14)               |       |
| 5–9                         |                      |                  |       |                          |                       |       |
| 10–14                       | 46 (132)             | 48 (108)         | 0.76  | 52 (25)                  | 49 (19)               | 0.59  |
| 15–17                       | 16 (46)              | 15 (31)          |       | 8 (4)                    | 13 (6)                |       |
| Mean age at diagnosis (years) | 11.2 ± 3.4          | 10.5 ± 3.4       | 0.39  | 10.8 ± 3.0               | 11.5 ± 4.4            | 0.32  |

Data are % (n) and means ± SD unless otherwise indicated.
of samples to be typed at one time compared with the prior assay (17,18). Previous validation studies based on OneLambda Luminex SSO typing yielded similar results when compared with the earlier SSO assay (19).

Genotypes assessed in both time periods were categorized as high risk (DRB1*03-DQB1*02/DRB1*04-DQB1*03); moderate to low risk (DRB1*04-DQB1*03/DRB1*04-DQB1*03, DRB1*03-DQB1*02/X†, DRB1*04-DQB1*03/unknown); low risk (DRB1*04-DQB1*03/DRB1*04-DQB1*03, DRB1*03-DQB1*02/X†, DRB1*03-DQB1*03); moderate to low risk (DRB1*03-DQB1*02/DRB1*04-DQB1*03, DRB1*04-DQB1*03/X†, DRB1*03-DQB1*03/unknown); and neutral risk (X/X, X/unknown). The X haplotype denotes all other haplotypes not defined above. The unknown haplotype denotes a haplotype that was unable to be typed.

Statistical analyses

Descriptive univariate analysis was used to determine the frequency of the genotypes for each period. χ² statistics were used to compare frequencies of HLA haplotypes by race and period and genotypes by high-risk (DRB1*03-DQB1*02/DRB1*04-DQB1*03) versus all other genotypes. Multivariate logistic regression was used to adjust for potential confounders (onset age, sex, family history of diabetes, and race/ethnicity) and assess temporal trends. High-risk was compared with all other genotypes using all other genotypes as the referent group. SAS version 9.1 (20) was used for all analyses.

RESULTS — A total of 364 youth (100 in 1978–1988, and 264 in 2002–2004) with new-onset type 1 diabetes at age 5–17 years had HLA genes measured as described above.

Table 2 shows the frequency of HLA class II DRB1-DQB1 genotypes by time period and ethnicity. More children (both NHW and Hispanic) carried the high-risk genotype (DRB1*03-DQB1*02/DRB1*04-DQB1*03) than in 1978–1988 (P = 0.05). Conversely, more children in 2002–2004 carried the moderate to low risk DRB1*04-DQB1*03 (homo- or heterozygous) genotype than in 1978–1988 (P = 0.04). There was no significant difference by time period or ethnicity in frequency of the neutral risk, DRB1*03-DQB1*02 (homo- or heterozygous), or X/X genotypes. These patterns were similar for NHW and Hispanic youth with type 1 diabetes.

Using multiple logistic regression analysis (Table 2), we assessed the association between having the high-risk genotype (versus all other genotypes) and time period, controlling for age, sex, race/ethnicity, and family history of diabetes. There was a 40% lower odds for carrying the high-risk genotype in 2002–2004 versus 1978–1988 (odds ratio 0.60 [95% CI 0.36–0.99], P = 0.05). A decreasing trend over the two time periods was noted for both NHWs (13%) and Hispanics (6%). Conversely, there was a 70% greater chance of carrying the moderate to low-risk genotypes in 2002–2004 versus 1978–1988 (1.7 [1.1–2.8], P = 0.03). No significant interactions between ethnicity and time period on the odds of having the highest risk genotype were noted. When stratified according to age-group (Table 3), the most dramatic decreasing trend in the prevalence of the high-risk genotype occurred in those aged 5–9 years (51% [1978–1988] vs. 32% [2002–2004], P = 0.05) with a coincident increasing trend in the proportion with the DRB1*04-DQB1*03 (homo- or heterozygous) genotype in the same age-group (19% [1978–1988] to 40% [2002–2004], P = 0.03). No significant differences over time were noted in the other age-groups.

CONCLUSIONS — We found that the distribution of HLA genotype changed in youth with type 1 diabetes diagnosed 27 years apart. Having type 1 diabetes at age 5–17 years in 2002–2004 was associated with an 11% difference or 0.6-fold lower odds for carrying the high-risk HLA genotype (DRB1*03-DQB1*02/DRB1*04-DQB1*03) compared with having type 1 diabetes in 1978–1988. The largest proportional difference (19%) over time in the high-risk genotype was

### Table 2 — Distribution of HLA class II genotypes (DRB1-DQB1) and odds ratios (ORs) for the association between genotype and period (1978–1988 vs. 2002–2004), according to participants’ ethnicities

|                  | NHW       | Hispanic  | All        |
|------------------|-----------|-----------|------------|
|                  | n (%)     | OR (95% CI)* | n (%)     | OR (95% CI)* | n (%)     | OR (95% CI)* |
| DRB1*03-DQB1*02/DRB1*04-DQB1*03 |          |           |            |             |            |
| 1978–1988        | 24 (41)   | 0.6 (0.3–0.99) | 15 (37)   | 0.8 (0.3–1.9) | 39 (39)   | 0.6 (0.4–0.99) |
| 2002–2004        | 63 (28)   | 1.0 (0.6–1.6) | 12 (31)   | 0.8 (0.3–1.9) | 75 (28)   | 1.0 (0.6–1.6)  |
| DRB1*04-DQB1*03/DRB1*04-DQB1*03, DRB1*04-DQB1*03/unknown |          |           |            |             |            |
| 1978–1988        | 18 (31)   | 1.7 (0.9–3.0) | 12 (29)   | 1.5 (0.6–3.8) | 30 (30)   | 1.7 (1.1–2.8)  |
| 2002–2004        | 96 (43)   | 1.0 (0.6–1.6) | 15 (38)   | 1.5 (0.6–3.8) | 111 (42)  | 1.0 (0.6–1.6)  |
| DRB1*03-DQB1*02/DRB1*03-DQB1*02, DRB1*03-DQB1*02/X†, DRB1*03-DQB1*03 |          |           |            |             |            |
| 1978–1988        | 12 (20)   | 1.1 (0.6–2.3) | 8 (19)    | 0.6 (0.2–2.0) | 20 (20)   | 1.1 (0.6–1.9)  |
| 2002–2004        | 50 (22)   | 1.1 (0.6–2.3) | 5 (13)    | 0.6 (0.2–2.0) | 55 (21)   | 1.1 (0.6–1.9)  |
| X/X, X/unknown   | 1978–1988 | 5 (8)      | 1.3 (0.4–4.2) | 7 (18)   | 1.3 (0.4–4.2) | 23 (8.7) | 0.8 (0.4–1.6)  |
| 2002–2004        | 16 (7)    | 0.8 (0.3–2.4) | 6 (15)    | 1.3 (0.4–4.2) | 23 (8.7) | 0.8 (0.4–1.6)  |

*OR for the association between period 2002–2004 versus 1978–1988 and carrying the high-risk genotype compared with all other genotypes. **DRB1*03-DQB1*02/DRB1*04-DQB1*03 not included. Data in bold are statistically significant.
found in 5- to 9-year-olds, suggesting that environmental pressures may have a greater impact on type 1 diabetes risk at earlier ages. This is the first documentation of such a trend in a representative biracial sample of youth with type 1 diabetes in the U.S.

Our study supports previous findings from Finland (9) and the U.K (8), both including Caucasian adults with childhood-onset type 1 diabetes diagnosed up to 80 years apart. The U.K. study showed that the frequency of the high-risk genotype was 12% lower in adults diagnosed up to 15 years of age in 1985–2002 compared with those diagnosed between 1922–1946 and 21% lower in those diagnosed under 5 years of age (8). The Finnish study showed that the frequency of the high-risk genotype was 7.1% lower and that of protective genotypes was 7.2% higher in adults with childhood-onset type 1 diabetes diagnosed after 1990 compared with those diagnosed before 1965 (9). Two older studies conducted in Finland in the 1980s reported a decrease in HLA-DR3 by 25% for those diagnosed with type 1 diabetes in the 1980s compared with those diagnosed in the 1960s (6,7). However, the major limitation of these studies was the potential for selection bias. Neither of these studies provided information on the size or representativeness of the referent population. More importantly, two studies (8,9) measured HLA in adults who were diagnosed with type 1 diabetes as children in the first half of the 20th century, when early mortality associated with type 1 diabetes was high. Therefore, the frequency of HLA genotypes in this sample may be significantly influenced by factors associated with survival of type 1 diabetes. An underestimate of the proportional distribution of high-risk HLA genotypes in earlier time periods (due to a potential association of high-risk HLA genes with increased mortality) may have resulted in underestimating the true temporal trends in the proportion of type 1 diabetes cases with high-risk HLA genotypes in earlier studies. In contrast, our study genotyped youth with type 1 diabetes relatively close to their disease onset and thus is not affected by survivor bias. In addition, we were able to provide evidence that the sample included in the analysis was representative of the Colorado population of youth with type 1 diabetes in both time periods.

Nevertheless, our study has several limitations. Importantly, although the same method was used for typing, due to the specific time periods in which they were performed, two slightly different assays were used, namely, OneLambda Lab-Type Luminex SSO in 2002–2004 and OneLambda SSO in 1978–1988. We were unable to validate the results obtained with the earlier SSO assay due to the lack of stored DNA samples and inability to recontact participants. However, previous studies (19,21) suggest comparable agreement in serologic specificity with an 85% allele agreement. Due to this and the difference in the number of allele SSOs available for typing over time, only the gene locus was used in this analysis. By not using expanded allele information, we potentially introduced some misclassification, likely increasing the proportion with the high-risk genotype. However, misclassification would be similar for both time periods and therefore unlikely to influence our analysis of trends. Because for 2002–2004 we did not have allele information on 100 DQB1 SSOs, we estimated the magnitude of the potential misclassification, and only four (1.5%) cases were misclassified as having the highest-risk genotype in 2002–2004 using the expanded allele information only. In addition, there were 12 youth from 1978–1988 that did not have complete genotypes. These youth were included in the study and classified as haplotype unknown. This categorization could have led to some misclassification. Specifically, if the unknown haplotype was DRB1*03-DQB1*02 or DRB1*04-DQB1*03, youth would have been classified as high-risk based on the typed haplotype or as heterozygous for the above haplotypes. For example, in the extreme situation where all youth with the unknown haplotype in the first time period would have been in fact at “high-risk,” the proportion of youth with the

### Table 3—Distribution of HLA class II genotypes (DRB1-DQB1) and odds ratios (ORs) for the association between genotype and period (1978–1988 vs. 2002–2004) by age-group at diagnosis

| Age Group          | 5–9 years of age | 10–14 years of age | 15–17 years of age |
|-------------------|-----------------|--------------------|--------------------|
| n (%)             | OR (95% CI)*    | n (%)             | OR (95% CI)*        | n (%)             | OR (95% CI)*        |
| DRB1*03-DQB1*02/DRB1*04-DQB1*03 |  |  |  |  |  |
| 1978–1988         | 19 (51)         | 0.9 (0.4–1.8)     | 6 (40)             | 0.5 (0.1–1.7)     |
| 2002–2004         | 32 (32)         | 0.45 (0.2–0.9)    | 34 (27)            | 2.9 (1.2–7.3)     |
| DRB1*04-DQB1*03/DRB1*04-DQB1*03, DRB1*04-DQB1*03/X†, DRB1*04-DQB1*03/unknown |  |  |  |  |  |
| 1978–1988         | 7 (19)          | 1.1 (0.4–2.8)     | 20 (42)            | 1.0 (0.2–5.9)     |
| 2002–2004         | 40 (40)         | 2.9 (1.2–7.3)     | 55 (43)            | 3.0 (0.7–12.6)    |
| DRB1*03-DQB1*02/DRB1*03-DQB1*02, DRB1*03-DQB1*02/X†, DRB1*03-DQB1*02/unknown |  |  |  |  |  |
| 1978–1988         | 7 (19)          | 1.1 (0.4–2.8)     | 9 (19)             | 0.6 (0.6–2.6)     |
| 2002–2004         | 20 (20)         | 0.6 (0.2–2.3)     | 28 (22)            | 4 (27)            |
| X/X, X/unknown   | 4 (11)          | 0.6 (0.2–2.3)     | 5 (10)             | 2 (13)            |
| 1978–1988         | 7 (8)           | 1.1 (0.4–2.8)     | 11 (8)             | 0.6 (0.6–2.6)     |
| 2002–2004         | 7 (8)           | 0.6 (0.2–2.3)     | 11 (8)             | 1.0 (0.2–5.9)     |

*OR for the association between period 2002–2004 versus 1978–1988 and carrying the high-risk genotype compared with all other genotypes. †DRB1*03-DQB1*02/DRB1*04-DQB1*03 not included. Data in bold are statistically significant.
References

1. Karvonen M, Viik-Kajander M, Moltchanova E, Libman I, LaPorte R, Tuomilehto J: Incidence of childhood type 1 diabetes worldwide: Diabetes Mondiale (Diab Mond) Project Group. Diabetes Care 23: 1516–1526, 2000

2. Vehik K, Hamman RF, Lezotte D, Norris JM, Klingensmith G, Bloch C, Rewers M, Dabelea D: Increasing incidence of type 1 diabetes in 0- to 17-year-old Colorado youth. Diabetes Care 30:503–509, 2007

3. Pociot F, McDermott MF: Genetics of type 1 diabetes mellitus. Genes Immun 3:235–249, 2002

4. Sheehy MJ, Scharf SJ, Rowe JR, Neme de Gimenez MH, Meske LM, Erlich HA, Nepom BS: A diabetes-susceptible HLA haplotype is best defined by a combination of HLA-DR and -DQ alleles. J Clin Invest 83: 830–833, 1989

5. Rotter JJ, Anderson CE, Rubin R, Congleton JE, Terasakl PI, Rimoin DL: HLA genotypic study of insulin-dependent diabetes the excess of DR3/DR4 heterozygotes allows rejection of the recessive hypothesis. Diabetes 32:169–174, 1983

6. Konttinen S, Scheinin T, Schlenzka A, Maenpaa J, Groop L, Koskimies S: Differences in HLA types in children with insulin-dependent diabetes diagnosed in 1960s, 1970s, and 1980s. Lancet 2:219, 1988

7. Maenpaa A, Koskimies S, Scheinin T, Schlenzka A, Akerblom HK, Maenpaa J, Reunanen A, Kontiainen S: Frequencies of HLA-DR, -DQ, -B8 and -Bw62 in diabetic children diagnosed between 1960 and 1990. Diabetes Res 16:159–163, 1991

8. Gillespie KM, Bain SC, Barnett AH, Bingley PJ, Christie MR, Gill GV, Gale EA: The rising incidence of childhood type 1 diabetes and reduced contribution of high-risk HLA haplotypes. Lancet 364:1699–1700, 2004

9. Herrmann R, Knip M, Veijola R, Simell O, Laine AP, Akerblom HK, Groop PH, Forsblom C, Pettersson-Fernholm K, Ilenon J: Temporal changes in the frequencies of HLA genotypes in patients with type 1 diabetes: indication of an increased environmental pressure? Diabetologia 46: 420–423, 2003

10. Hamman RF, Gay EC, Cruickshanks KJ, Cook M, Lezotte DC, Klingensmith GJ, Chase HP: Colorado IDDM Registry: incidence and validation of IDDM in children aged 0–17 yr. Diabetes Care 13:499–506, 1990

11. Kostraba JN, Gay EC, Cai Y, Cruickshanks KJ, Rewers MJ, Klingensmith GJ, Chase HP, Hamman RF: Incidence of insulin-dependent diabetes mellitus in Colorado. Epidemiology 3:232–238, 1992

12. SEARCH for Diabetes in Youth Study Group: SEARCH for Diabetes in Youth: a multicenter study of the prevalence, incidence and classification of diabetes mellitus in youth. Control Clin Trials 25:458–471, 2004

13. Verlato G, Muggeo M: Capture-recapture method in the epidemiology of type 2 diabetes: a contribution from the Verona Diabetes Study. Diabetes Care 23:759–764, 2000

14. Cochi SL, Edmonds LE, Dyer K, Greaves WL, Marks J, Rovira EZ, Preblud SR, Orenstein WA: Congenital rubella syndrome in the United States, 1970–1985: on the verge of elimination. Am J Epidemiol 129:349–361, 1989

15. Cruickshanks KJ, Jobim LF, Lawler-Heavener J, Neville TG, Gay EC, Chase HP, Klingensmith G, Todd JA, Hamman RF: Ethnic differences in human leukocyte antigen markers of susceptibility to IDDM. Diabetes Care 17:132–137, 1994

16. Zetterquist H, Olerup O: Identification of the HLA-DRB1*04, -DRB1*07, and -DRB1*10 allele by PCR amplification with sequence-specific primers (PCRSSP) in 2 hours. Hum Immunol 34:64–74, 1992

17. Blair L, Nong T, Manzo A, Chamblin J, Neswald K, Bedrossian A, Berman D, Liu A: A new reversed SSO HLA (class I and II) DNA typing method using fluorescently labeled microspheres and flow analyzer (Abstract). Eur J Immunogenet 29: 2002

18. Saito K, Lee J, Blair L, Nong T, Chamblin J, Neswald K, Bedrossian A, Berman D, Liu A, Denham S: A new reversed SSO HLA (-DR) typing method using fluorescently labeled microspheres and flow analyzer (Abstract). Hum Immunol 62:2001

19. Geralomi K, Lorber M: HLA-DR typing via LABType and the Luminex LabMAP (Abstract). Hum Immunol 62:2001

20. SAS/STAT software: changes and enhancements. Release 9.1, 2001. Cary, NC, SAS Institute Inc.

21. Ossowski L, Jakubek J, Woronkowicz M, Littleton N, KuKuruga D: The Luminex Microbead Array Assay is an excellent sequence specific oligonucleotide probe (SSOP) method for HLA-A, B and DR antigen level typing (Abstract). Hum Immunol 62:2001

22. Wilkin TJ: The accelerator hypothesis: weight gain as the missing link between type I and type II diabetes. Diabetologia 44:914–922, 2001

23. Punziate-Lycca A, Dahlquist G, Nystrom L, Arnegust H, Bjork E, Bohlme G, Bolinder J, Eriksson JW, Sundkvist G, Ostman J: The incidence of type 1 diabetes has not increased but shifted to a younger age at diagnosis in the 0–34 years group in Sweden 1983 to 1998. Diabetol 45:783–791, 2000

24. Gale EA: A missing link in the hygiene hypothesis? Diabetes 45:588–594, 2002