CLINICAL STUDY

HLA-DR genotypes influence age at disease onset in children and juveniles with type 1 diabetes mellitus

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Abstract

Objective: To investigate HLA-DR genotype in association with chronological age or calendar year of disease onset and the time trend of genotype frequencies from 1969 to 2009. Additionally, to examine genotype frequency in relation to B-cell-, islet cell antibodies (ICA)-, autoantibodies to insulin-, insulinoma antigen 2 (IA2)-, glutamic acid decarboxylase-antibody positivity, thyroid antibody positivity, thyroid diseases or coeliac antibody positivity. Genotype associations with gender and ethnicity are also analyzed.

Subjects and methods: HLA-typed children and juveniles ($n=1445$) aged ≤ 20 years at disease onset from the German/Austrian DPV-database were examined. χ^2 statistics and mixed hierarchical logistic regression models were used to compare genotype frequencies and establish associations with age at disease manifestation, autoimmune antibodies/diseases, ethnicity and time trend.

Results: Subjects aged < 6 years predominantly carried the genotype HLA-DR3/4 (25.2%), whereas in subjects aged > 12 years the most prevalent HLA-DR genotype was X/X (18.1%). IA2 positivity was associated with HLA-DR4/X and HLA-DR3/4 positivity ($P=0.011$), and hypothyroidism was linked to HLA-DR4/4 ($P=0.0103$). More females carried the HLA-DR4/4 genotype (18.2 vs 12.7% $P=0.0048$) or were thyroid antibody positive (24.5 vs 14.7% $P=0.0001$). Larger numbers of < 6 year olds were coeliac antibody positive than > 12 year olds (14.8 vs 9.1% $P=0.0037$). No associations between migration background and B-cell-, thyroid- or coeliac-antibody positivity, and no time trend were found.

Conclusion: HLA-DR genotype associated with age at disease onset, ICA positivity and hypothyroidism; female gender with thyroid antibody positivity and low age of diabetes onset with coeliac antibody positivity.

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Introduction

Worldwide, the incidence of autoimmune type 1 diabetes mellitus (T1DM), resulting from the destruction of insulin-producing β -cells in pancreatic islets, is increasing steadily especially in the age group < 20 years (1, 2). This disease often manifests during childhood with a peak between the ages of 10 and 14 years (3). However, T1DM onset in adulthood is also possible (4). Even though genetically determined susceptibility accounts for at least half of the lifetime risk of T1DM manifestation (5), the observed increases in prevalence suggest that environmental factors have a growing impact (6). Hence, a changing 'diabetogenic' milieu may modify the impact of risk genes due to gene–environment interplay.

Previous research indicates that progression to disease and age at onset are directly linked to the MHC II on chromosome 6p21.3 (7). In at least 50% of cases, the inheritance of susceptibility and resistance are predicted by specific HLA-DRB1 and HLA-DQB1 alleles (HLA-DR and HLA-DQ genotypes). HLA-DR3 and HLA-DR4 are predisposing genotypes, and susceptibility of heterozygous individuals (HLA-DR3/DR4) is higher than that of any other genetic constellation (8, 9). Individuals with the genotypes HLA-DR3/X, HLA-DR4/X, HLA-DR3/3 and HLA-DR4/4 have a moderate risk of developing disease, while intermediate/'neutral' risk is attributed to non-DR3/non-DR4 genotypes (HLA-DRX/X) with the lowest risk of developing disease. Susceptibility may however vary between ethnic groups and geographical regions (10, 11).

In order to examine HLA-DR genotypes over time, a large sample of subjects from the German/Austrian multicentre documentation (DPV) database was investigated from 1969 to 2008. Associations between HLA-DR genotype frequencies, age at disease onset, gender, ethnicity and autoimmune disease markers such as thyroid antibody positivity, coeliac antibody positivity as well as autoimmune diseases such as hypothyroidism or hyperthyroidism (12, 13) were studied. Considering that about 80% of people with T1DM are positive for at least one islet cell antibody (ICA) (14), the frequencies of HLA-DR genotypes were additionally examined in relation to B-cell- (ICA-, autoantibodies to insulin (insulin autoantibodies (IAA))- , insulinoma antigen 2 (IA2)- and glutamic acid decarboxylase (GAD)-) antibody positivity. Antibody associations with age at disease onset and gender were also examined.

Thus, the aim of this work is to examine associations between: age at disease onset and HLA-DR genotype; gender, ethnicity and HLA-DR genotype; ICA positivity and HLA-DR genotypes; thyroid antibody positivity, hypothyroidism, hyperthyroidism and HLA-DR genotypes; coeliac antibody positivity and HLA-DR genotypes; age at disease onset, sex, thyroid- and coeliac-antibody positivity; changes in HLA-DR genotype frequencies from 1969 to 2008 (linear time trend).

Subjects and methods

The multicentre DPV-database which collates data of patients treated for diabetes from 304 DPV-centres (outpatient or inpatient health facilities, university teaching hospitals, general hospitals, specialized diabetes treatment centres or general practitioners offices) in Germany or Austria was used to identify the study cohort. Participating centres transmit anonymous, standardized, prospective data twice a year to the central administrative unit in Ulm (Germany) for evaluation and benchmarking. In order to ensure optimal data validity, all inconsistent data are reported back to the respective centres for correction every 6 months (15). Sixty-three DPV-centres contributed genotyped patients to this study. The sample consisted of 1445 HLA-typed T1DM patients, selection criterion: disease onset at age ≤ 20 years. The cohort included subjects with a migration background. Migration background was defined as father or mother born outside Germany or Austria respectively.

Genotypes

Risk of T1DM based on HLA-DR genotypes can be categorized as follows: highly predisposing, predisposing, intermediate (neutral), protective or very protective (10). Genotypes were categorized as follows: HLA-DR3/4 (DRB1*03-DQA1*0501-DQB1*0201-/DRB1*04-DQA1*0301-DQB1*0302) (highly predisposing);

Table 1 Classification of genotypes in the study cohort.

DRB1–DQB1 haplotypes	DR3	DR4	DRX
DRB1	03	04	Neither 03 nor 04
DQA1	0501	0301	Neither 0501 nor 0301
DQB1	0201	0302	Neither 0201 nor 0302
HLA-DR3/4	+	+	–
HLA-DR4/4	–	+/+	–
HLA-DR4/X	–	+	+
HLA-DR3/3	+/+	–	–
HLA-DR3/X	+	–	+
HLA-DRX/X	–	–	+/+

HLA-DR genotypes: for the purpose of simplicity, genotypes were summarized as follows: HLA-DR3/4, DR4/4, DR4/X, DR3/3, DR3/X and DRX/X. Haplotype present (+); haplotype absent (–).

HLA-DR4/4, HLA-DR3/3, HLA-DR4/X, HLA-DR3/X (predisposing); and non-DR3 and non-DR4 genotypes (HLA-DRX/X) encompassed intermediate/‘neutral’/protective genotypes. Table 1 shows the classification of genotypes in this DPV-study sample.

Genotyping of HLA-DRB1 and DQB1 In the 1970s and 1980s, serological typing was used to establish HLA-DR and DQ alleles for subjects in the DPV-database. In the 1990s, HLA typing was performed using polymerase chain amplification with sequence-specific priming methods. Comparability of results from different laboratories was ensured through the use of standard assays for HLA-typing and the official nomenclature for factors of the HLA System, 1991.

Autoantibodies

B-cell antibodies The following β -cell autoantibodies were analyzed: anti-IAA (measured at diagnosis), anti-GAD, anti-ICA and anti-IA2 antibodies. The following cut-off values were defined in order to classify positivity: IA2 (≥ 0.75 Units/ml); IAA at diagnosis (≥ 1 Unit); ICA (≥ 5 JDF Units); GAD (≥ 0.9 Units/ml).

Thyroid antibodies Anti-thyroperoxidase (anti-TPO) antibodies and anti-thyroglobulin (anti-TG) antibodies were measured using commercially available RIAs at different laboratories. For both anti-TPO and anti-TG, positivity was assumed if a titre exceeding 100 U/ml was measured. Each anti-TPO-assay was calibrated using the MRC 66/387 anti-TPO standard.

Coeliac-specific antibodies Positivity for coeliac antibodies was defined by gliadin IgG and IgA values above 25 U/l. Given that a coeliac disease diagnosis based on IgG anti-gliadin antibody positivity is unspecific, a positive titre of IgA autoantibodies directed against tissue transglutaminase (anti-tTG antibodies) or endomysium antibodies (EMA) higher than 10 U indicated that coeliac disease could be suspected.

Statistical analysis

Data analysis was carried out using the Statistical Analysis Software (SAS, Cary, NC, USA). χ^2 statistics were used to compute genotype frequencies in relation to gender, age category of disease onset (<6, 6–≤12 and >12 years), migration background, disease manifestation year, B-cell antibody positivity, ICA positivity, thyroid antibody positivity, hypothyroidism, hyperthyroidism and coeliac antibody positivity. Mixed hierarchical logistic regression models were used to examine associations between genotype and age at disease onset adjusted for sex, manifestation year and treatment centre (random effect). Genotype frequency was also analyzed in association with thyroid- and coeliac-antibody positivity adjusted for age at disease onset, sex, manifestation year and treatment centre (random effect). Adjusted means of covariates are presented in Figs 1–4. The time trend was investigated as follows: i) comparing genotype frequency in the periods 1969–1989 and 1999–2009, and ii) comparing genotype frequency from 1969 to 2009 in 5-year intervals. The period from 1969 to 1978 was summarized in one (10 years) interval, due to the low number of HLA-typed cases documented during these years. Logistic regression models were used to assess genotype frequency over time in both categories, adjusted for age at disease onset, sex and treatment centre.

Figure 1 Age at disease onset. Frequency of HLA-DRX/X and HLA-DR3/4 genotypes in three age categories (<6, 6–≤12 and >12 years) adjusted for sex and manifestation year (logistic regression).

Figure 2 Gender differences. HLA-DR4/4 genotype frequency among females and males adjusted for age and manifestation year (logistic regression).

Results

Study population

The sample consisted of HLA-typed T1DM patients ($n=1445$), aged ≤20 years at disease onset. Fifty per cent of patients in the cohort were diagnosed after 1992, while 24.6% were diagnosed after 2000. There were only minimal differences between HLA-typed patients and non-HLA-typed patients ($n=46\,287$) in the database. Male gender was 50.7 vs 51.8%, and age at disease onset was 8.3 vs 8.7 years for HLA-typed and non-HLA-typed subjects respectively. In the study cohort, 485 patients were <6 years old, 647 patients were 6–≤12 years old and 313 patients were 12–≤20 years old. Table 2 shows demographic variables pertaining to the study cohort. There were 7.7% patients with migration background in the study sample.

Frequency of genotypes

In the study sample, the moderate-risk genotypes (HLA-DR4/X (29.0%), HLA-DR4/4 (15.8%), HLA-DR3/X (10.7%) and HLA-DR3/3 (8.5%)) constituted

Figure 3 Immunologic differences and gender. Thyroid antibody positivity among females and males adjusted for age at disease onset, genotype and manifestation year (logistic regression).

Figure 4 Immunologic differences and age categories. Significant differences in coeliac antibody positivity in different age categories, adjusted for sex, genotype and manifestation year (logistic regression).

the most abundant category, together accounting for 64% of all genotypes. The second most abundant category was the high-risk genotype, HLA-DR3/4, with 24.8%. The lowest risk category, HLA-DRX/X, was carried by 11.1% of the sample. The high-risk group HLA-DR3/4 consisted of 145 subjects <6 years old, 153 patients 6–≤12 years old and 61 patients 12–≤20 years old. In the low-risk group, HLA-DRX/X, 43 subjects were <6 years old, 68 subjects were between 6 and 12 years old and 50 subjects were >12 years old. The frequencies of genotypes analyzed are presented in Table 3.

Age at disease onset and HLA-DR genotypes

Age at disease onset was significantly related to HLA-DR genotype after adjusting for sex and manifestation year in the study sample ($P=0.0067$). A closer look at age/genotype associations within the three categories, i.e. <6 years ($n=485$), 6–≤12 years ($n=647$) and >12 years ($n=313$) revealed that both HLA-DR3/4 and HLA-DRX/X genotypes were significantly associated with age at disease manifestation. In total, 25.2% of subjects in the age category <6 years, 20.1% in the age category 6–≤12 years and 16.4% in the age category >12 years carried the HLA-DR3/4 genotype. Analogously, the HLA-DRX/X genotype was carried by 18.1% of subjects in the age category >12 years, while

the 6–≤12 years and <6 years age categories consisted of 12 and 10.3% of subjects with HLA-DRX/X respectively. HLA-DR3/4 was associated with the lowest age at disease onset (6.8 ± 0.3 years), while HLA-DRX/X was linked to the highest age at disease onset (8.2 ± 0.4 years) in the study sample (Fig. 5). Significant differences in age at disease onset for subjects with HLA-DR3/4 and HLA-DRX/X genotypes are illustrated in Fig. 1. The moderate-risk genotypes (HLA-DR3/3, HLA-DR3/X, and HLA-DR4/X, HLA-DR4/4) showed no significant associations with age category at disease manifestation.

Gender, ethnicity and HLA-DR genotype

In the study cohort, there were more males than females with the HLA-DR3/3 (56.1 vs 43.9%), HLA-DR3/4 (53.5 vs 46.5%), HLA-DR4/X (54.9 vs 46.1%) and HLA-DRX/X (51.6 vs 48.4%) genotypes respectively. Inversely, more females than males had the HLA-DR3/X (55.5 vs 44.5%) and HLA-DR4/4 (59.2 vs 40.8%) genotypes. The significant gender difference in genotype frequency ($P=0.0078$) was lost after adjusting for age at disease onset and manifestation year for all genotypes except HLA-DR4/4. In this subgroup, there were more females (18.2%) than males (12.7%), $P=0.0048$ (Fig. 2).

Genotype frequencies did not significantly differ between subjects with and without a migration background.

ICAs and HLA-DR genotypes

B-cell antibody positivity was tested in 1073 (74.3%) of patients in the study cohort, and 811 (75.6%) were found positive for any B-cell antibody. There was no significant association between HLA-DR genotypes and ICA positivity in general. HLA-DR genotype associations with individual ICAs (i.e. ICA, IAA, GAD and IA2) revealed a significant association for IA2 only ($P=0.0119$). More subjects with HLA-DR3/4 and HLA-DR4/X genotypes were IA2 positive than IA2 negative, i.e. HLA-DR3/4 (57 vs 42) and HLA-DR4/X (68 vs 37) respectively. Conversely, more IA2-negative subjects than IA2-positive subjects carried the HLA-DR3/X genotype (32 vs 15). All other genotypes had comparable frequencies between IA2-positive and IA2-negative subjects.

Table 2 Demographic characteristics of the study cohort.

Demographic variables	
Number examined	1445
Sex (males/females): frequency (%)	50.7/49.3
Mean age at diagnosis: years $\pm$ s.d.	8.3 $\pm$ 4.2
Migration background: frequency (%)	7.7

Sample characteristics: frequency (%) of categorical variables; mean and s.d. of continuous variable.

Thyroid antibody positivity, thyroid disease and HLA-DR genotype

Thyroid antibodies were tested in 1182 (81.8%) subjects of the study sample, and 294 (24.9%) were positive. No significant association was found between thyroid antibody positivity and HLA-DR genotype. There was however, an association between HLA-DR

Table 3 HLA-DR genotypes and corresponding antibody profiles.

Frequency examined (n=1445)	Proportion (%)	Antibody profile	Frequency examined	Proportion positive (%)
HLA-DR genotypes				
HLA-DR3/3 (n=123)	8.5	Any B-cell antibody	85	74.1
		Thyroid antibody	89	28.1
		Coeliac antibody	91	8.71
HLA-DR3/4 (n=359)	24.8	Any B-cell antibody	279	72.4
		Thyroid antibody	299	28.1
		Coeliac antibody	284	16.1
HLA-DR3/X (n=155)	10.7	Any B-cell antibody	131	68.7
		Thyroid antibody	137	24.1
		Coeliac antibody	126	15.8
HLA-DR4/4 (n=228)	15.8	Any B-cell antibody	160	75.0
		Thyroid antibody	168	22.6
		Coeliac antibody	166	16.7
HLA-DR4/X (n=419)	29	Any B-cell antibody	309	80.9
		Thyroid antibody	356	22.5
		Coeliac antibody	319	12.5
HLA-DRX/X (n=161)	11.1	Any B-cell antibody	109	78.9
		Thyroid antibody	133	25.6
		Coeliac antibody	117	14.5

Genotype frequency (%) and corresponding antibody profile. Frequency (%) examined and proportion (%) positive for respective antibodies.

genotype and clinically diagnosed hypothyroidism ($P=0.0103$) but not hyperthyroidism ($P=0.6305$). Among the 30 subjects diagnosed with hypothyroidism, 12 carried the HLA-DR4/4 genotype and 9 carried the HLA-DR4/X genotype. Less than five individuals carrying other genotypes were diagnosed with hypothyroidism in the study sample.

Coeliac antibody positivity and HLA-DR genotype

In the study sample, 1103 (76.3%) subjects were examined for coeliac antibodies, and 168 (15.2%) were positive (Table 4); of the 1439 subjects examined, 3.5% had clinically diagnosed coeliac disease. No significant association was found between HLA-DR genotype and coeliac antibody positivity. Thus, among patients of the study sample, coeliac antibody positivity was independent of HLA-DR genetic make-up.

Immunological phenotypes in association with gender and age at disease onset

A significant gender difference was found with regard to thyroid antibody positivity. More females (24.5%) than males (14.7%) remained positive after adjusting for genotype and calendar year of disease manifestation ($P=0.001$), Fig. 3. There was no significant association between gender and B-cell- or coeliac-antibody positivity.

However, the presence of coeliac antibodies was significantly associated with age at disease onset. Coeliac antibody-positive subjects were predominantly found in the age category <6 years (14.8%) followed by the age categories $6\text{--}\leq 12$ years and >12 years with

10.5 and 9.1% of subjects respectively ($P=0.0037$; Fig. 4). There was no significant relationship between age category and B-cell antibody positivity.

HLA-DR genotypes: time trend from 1969 to 2009

Before adjusting for age at disease onset and sex, differences in genotype frequencies between 5-year intervals from 1969 to 2009 were significant ($P=0.001$). This was also true when genotype frequencies within the periods 1969–1989 and 1999–2009 were compared ($P=0.0056$). However, after adjusting for age at disease onset and sex, significance was lost for all genotypes for all intervals examined. Thus, no significant linear time trend persisted for any genotype over the 40 years of investigation in the study sample.

Figure 5 Age at disease onset. Mean (S.E.M.) age at disease onset for each genotype adjusted for sex, manifestation year and centre (random variable).

Table 4 Immunological phenotypes pertaining to the study cohort.

Antibody profile	Frequency examined	Proportion of sample (%)	Frequency positive	Positivity (%)	Association with HLA genotype (<i>P</i>)
Immunological phenotype sample <i>n</i> =1445					
Any B-cell antibody	1073	74.3	811	75.6	0.0643
GAD antibody	469	32.5	257	54.8	0.0763
ICA antibody	977	67.6	616	63.1	0.1363
IA2 antibody	355	24.5	195	54.9	0.0112*
IAA antibody (at diagnosis)	834	57.7	551	66.07	0.5566
Thyroid antibody	1182	81.8	294	24.9	0.5805
Coeliac antibody	1103	76.3	168	15.2	0.6594

Frequency (%) of antibodies examined and respective proportion (%) of antibody positivity. **P*<0.005.

Discussion

In this work, the frequencies of HLA-DR genotypes were examined over a period of 40 years in a large sample of patients from many German and Austrian health facilities. Associations between HLA-DR genotypes and age at onset, gender, markers of humoral immunity and autoimmune diseases were also investigated.

We observed that the distribution of genotypes within risk categories in the study cohort was as follows: high-risk genotype (24.8%), moderate-risk genotypes (64%) and low-risk genotype (11.1%). This distribution was similar to that of other Caucasian populations (16).

Previous studies on age-dependent HLA genetic heterogeneity of T1DM indicate that HLA-DR3/DR4 is highly predisposing to T1DM, and associates with a low age at disease onset (17–19). In this study, disease in patients with the HLA-DR3/4 genotype manifested at a significantly younger age (6.8 ± 0.3 years) than in patients with the HLA-DRX/X genotype who had the highest mean age at disease onset in the sample (8.19 ± 0.37 years). For the entire sample, mean age at disease manifestation was $8.3 (\pm 4.2)$ years. Thus, our results confirm the findings of previous studies whereby T1DM manifests at lower ages in individuals with the highly predisposing HLA-DR3/4 genotype and significantly later in patients with the intermediate/‘neutral’ risk HLA-DRX/X genotype (18). Associations between age at disease onset and the moderate-risk genotypes (HLA-DR3/3, HLA-DR4/4, HLA-DR4/X and HLA-DR3/X) were non-significant in this study.

An association between genotype and gender was found only within the HLA-DR4/4 subgroup, where more females than males carried this genotype. Female gender dominance of HLA-DR4/4 genotype in diabetes has not been previously reported. Currently, there is no evident reason for this female gender dominance for the HLA-DR4/4 genotype observed for this DPV-cohort. Nevertheless, this result may be an indication that HLA-DR genotypes have stronger gender associations in determining susceptibility to T1DM than previously assumed. Female gender dominance was also observed with regard to thyroid hormone positivity and hypothyroidism, suggesting a direct influence of the gender on the disease phenotype (20).

Even though ethnic variability in the incidence of diabetes has previously been reported (2), no association between HLA-DR genotype and ethnicity was observed in the study sample, and subjects with a migration background had comparable demographic characteristics to native subjects.

Alongside a general increase in the prevalence of T1DM, some studies have reported changes in the frequency of HLA-DR genotypes across time. These studies usually suggest that in recent years, individuals with childhood and youth onset diabetes have fewer classical high-risk HLA genes than peers diagnosed 40–60 years ago (9, 17). They usually noted a decrease in the frequency of high-risk HLA-DR genotypes, and a concomitant increase in low-risk genotypes over time in the respective populations examined, and underline the notion that genetic contribution towards the development of T1DM changes across time. However, in the present study, changes in genotype frequencies over 40 years in subjects aged ≤ 20 years at disease manifestation did not show any significant time trends. However, more HLA-typed subjects were registered in the DPV-data bank after 1999 than in the years before, i.e. between the mid-1990s and 2003; more HLA-typed subjects were documented for all genotypes than in the years before or after this period. This nevertheless did not lead to significance in the time trend of any genotype. The absence of significant time trends in this work is in line with recent analyses which suggest that the distribution of risk genes within the risk categories ‘high risk’, ‘moderate risk’ and ‘low risk’ has remained unchanged over several decades (17, 20). This absence of a continuous time trend could have been due to the influence of environmental pressures associated with T1DM, e.g. seasonal variations with related increased prevalence of enteroviruses caused illnesses in winter months than in the summer months (21, 22). Additionally, modern lifestyles of excessive calorie intake (increases in BMI), physical inactivity, shortened sleep, excessive hygiene or exposure to toxic substances (23) may have also contributed to this development. Nevertheless, environmental pressures and lifestyle may reduce the need for a strong genetic background for the development of T1DM (24).

An analysis of HLA-DR genotype and autoimmunity revealed that IA2 positivity was significantly associated with HLA-DR3/4 and HLA-DR4/X genotypes. Previous studies have reported associations between IA2 and HLA-DR genotypes with at least one HLA-DRB1* 0401 allele (25, 26). Our results confirm these findings, and underline a genotype-dependent association of IA-2 in the aetiology of T1DM. Even though IAA positivity has been associated with homozygosity of the HLA-DR4 genotype and male gender during adolescence (27), no similar association was found in the study sample. No significant associations were also found between HLA-DR genotype and GAD-, ICA- and B-cell-antibody positivity in general.

An association between HLA-DR genotype and autoimmune disorders was found for hypothyroidism, which was predominantly diagnosed in patients with the HLA-DR4/4 and HLA-DR4/X genotypes. This suggests that moderate-risk-associated HLA-DR genotypes also play a role in thyroid disease manifestation. Thyroid antibody positivity was not genotype related but gender (female) related. This significant female gender dominance for thyroid antibody positivity in the study sample has been previously reported (20, 28). Additionally, age at disease onset was significantly linked to coeliac antibody positivity in the sample. Subjects <12 years old were more likely to be positive for coeliac antibodies than older subjects in the study cohort. This age association has also been observed in previous studies (12).

Conclusion

This work, based on a large cohort of T1DM patients from two medium-risk European countries (Germany and Austria), shows that a lower age of disease onset (<6 years) is linked to the highly predisposing genotype (HLA-DR3/4), while the intermediate (neutral) genotype, HLA-DRX/X, is associated with higher age at disease manifestation (>12 years). Changes in genotype prevalence did not follow a linear trend across the 40-year period of investigation. Thus, despite the increase in incidence of T1DM in Germany/Austria, the frequency of HLA genotype risk categories has remained unchanged.

Study limitations and strengths

The absence of a linear time trend may have been due to the fact that a large number of subjects were examined for changes which are not immediately evident, e.g. environment-attributed influences. Since these were not examined in detail, the validity of the study findings for time trends may have been compromised. This may also explain the fluctuations in genotype frequency observed. Furthermore, it is possible that among patients with adult onset of disease, different genotype associations exist from disease onset during childhood.

Nevertheless, a major strength of this study is that the data used were collected in the standardized, long-term follow-up DPV-documentation process from a large sample. As such, a selection bias was unlikely. Nonetheless, similar studies over comparable periods are needed in order to backup the results of this analysis.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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