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Correlations between the incidence of childhood-onset Type I diabetes in Europe and *HLA* genotypes

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Abstract

There are large variations in the incidence of Type I (insulin-dependent) diabetes mellitus within Europe, ranging from 3.2 cases per 100000 person-years in the Republic of Macedonia to more than 40 new cases per 100000 person-years in Finland. This variation could be caused by differences in the distribution of genetic susceptibility markers, by differences in the distribution of environmental disease determinants or by a combination of both. To assess how much genes contribute to this variation, we correlated the level of incidence of Type I diabetes with the prevalence in the general population of genetic susceptibility and protective markers encoded by the human leukocyte antigen *(HLA)-DQ* loci. Positive association was found for the combined group of genotypes associated with Type I diabetes risk ($p < 0.001$). The whole positive effect was, however, accounted for by the *HLA-DQ2/DQ8* (DQA1*0501-DQB1*0201/DQA1*0301-DQB1*0302) and *HLA-DQ4/DQ8* (DQA1*0401-DQB1*0402/DQA1*0301-DQB1*0302) genotypes ($p < 0.001$ and $p < 0.004$, respectively). No correlation was found between incidence of Type I diabetes and population prevalence of genotypes not encoding for aspartate on position 57 on the HLA-DQ β chain. It was not possible to detect any negative correlation between Type I diabetes incidence and the prevalence of *HLA*-genotypes conferring protection against Type I diabetes in a population (HLA-DQA1*0102-DQB1*0602/X). The results suggest that a substantial part of the transnational variation in the incidence of childhood-onset Type I diabetes in Europe is explained by variations between populations in the distribution of particular DQ genotypes which confer a high risk of Type I diabetes in the general population. [Diabetologia (2001) 44 [Suppl 3]: B 51–B 59]

Keywords *DQ* genotypes, Europe, incidence, populations, Type I diabetes.

Recent research has identified two major features which influence the frequency and distribution Type I (insulin-dependent) diabetes mellitus in childhood. First, in the northern European countries a steady increase, about 3 % annually in incidence (and hence, disease risk), has been observed, during the last decades [1]. Second, on the global scale Europe has the highest risk of Type I diabetes that has been measured, ranging tenfold from Finland (highest) to South-eastern Europe (lowest) but with striking regional variations [1–3].

Type I diabetes is caused by the interaction of genetic factors and environmental factors. The rise in Type I diabetes incidence cannot be solely explained by genetic mechanisms but it is still not known whether the geographical variation in diabetes incidence is attributable to differences in the distributions of environmental factors or genes, or a combination of both. Correlating the degree of population risk of Type I di-

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Abbreviations: HLA, Human leukocyte antigen, Asp57, aspartic acid at DQβ-chain residue 57, Non-Asp57, amino acids other than aspartic acid at DQβ-chain residue 57.

* see Acknowledgments

Table 1. Incidence data used for analysis, by population

Country	Abbreviation	Number of incident cases	Estimated number of person-years at risk	Standardized incidence rats [a] (0–14 years) per 100000
Finland	FIN	1014	2872521	35.30
Sardinia	SARD	661	1843279	35.86
Sweden	S	3836	15721311	24.40
Denmark	DK	226	1381418	16.36
Norway	N	491	2316038	21.20
Northern Ireland	N IRE	462	2352342	19.64
England	UK	1373	8382173	16.38
The Netherlands	NL	422	3236196	13.04
Czech Republic	CZ	1113	12926829	8.61
Austria	A	754	8249453	9.14
Germany	D	1201	10544337	11.39
Spain	E	847	6847211	12.37
Belgium	B	112	966350	11.59
Luxembourg	LUX	49	405629	12.08
France	F	835	10048135	8.31
Portugal	P	76	507343	14.98
Italy	I	1079	13762755	7.84
Greece	GR	335	3511530	9.54
Israel	ISR	433	7389078	5.86
Poland	POL	1182	19003215	6.22
Hungary	HUN	821	9287330	8.84
Republic of Macedonia	MAC	93	2952381	3.15
Romania	ROM	138	2782258	4.96
Bulgaria	BUL	513	6349010	8.08
Russia	RUS	351	5441860	6.45
		18417	159079987	11.58

[a] Standardized incidence rate is based on groups matched for (0–4, 5–9 and 10–14 years) of equal size

abetes with the prevalence of genetic susceptibility markers in the general population might throw light on this issue.

About 20 genomic regions have been identified which could contribute to Type I diabetes development [4–6]. The *HLA* region on the short arm of chromosome 6 is the most important, accounting for 42 % of the familiar clustering of Type I diabetes [4]. Type I diabetes is most strongly associated with *HLA-DQA1* and *DQB1* genes [7–10]. Several models have been put forward to explain this association. One study suggested that alleles encoding amino acids other than aspartic acid at *DQβ* chain residue 57 (non-Asp57) are associated with Type I diabetes susceptibility [7]. Alleles encoding Asp57 appear to be associated with dominant protection [11]. However, other later studies indicate that the Type I diabetes association is more complex [12,13]. Investigators have proposed that Type I diabetes is primarily associated with DQ$\alpha\beta$ heterodimers composed of a DQα chain having arginine at residue 52 in combination with a non-Asp57 DQβ-chain [14, 15]. We have suggested that Type I diabetes could be primarily associated with five or six particular *cis*-encoded or trans-encoded DQ$\alpha\beta$ heterodimers [16,17]. The DQ$\alpha\beta$ heterodimer encoded by DQA1*0102 and DQB1*0602 (found on a *DR2* haplotype) seems to confer dominant protection [9,17,18]. Data from the 11th International Histocompatibility Workshop where 981 unrelated Type I diabetes patients and 2228 healthy control subjects were genomically *HLA* class II typed, revealed that the highest Type I diabetes risk among Caucasians is associated with six *DQ* genotypes [19]. Our ecological analysis tests associations between the *HLA-DQ* genotypes in a population with incidence rates of Type I diabetes.

Materials and methods

Type I diabetes incidence. New data on the incidence of Type I diabetes in childhood has been obtained from the EURODIAB studies [1, 2]. Our analysis uses data from the period 1989–1994 [1]. To increase statistical power, the incidence estimates from Finland and Sweden have been based on data covering their respective total populations [3]. The same sources also provided information on incidence in Russia. The incidence data which has formed the basis of our analyses is shown in Table 1.

Genotype data. The *HLA-DQA1* and *-DQB1* genotypes were available for 11 736 healthy control subjects (genomic marker contributors) for 25 of the countries participating in the EURODIAB ACE network. The HLA class II genotyping was done at the local laboratories of the various network centres and was mainly carried out by polymerase chain reaction with subsequent hybridisation to sequence-specific oligonucleotide probes (PCR-SSOP) according to the protocols of the 11th International *HLA* Workshop, while some centres used polymerase chain reactions with sequence-specific primers [20,21].

Table 2. *HLA-DQ* genotype data for control subjects, by population; absolute numbers (with genotype frequencies in brackets)

Country	Number of subjects	Strong	Weak	All	Rest	Max	*DQ8/DQ4*	Number of subjects	ASP-/ASP-	Number of subjects	*DQ6/X*
Finland	163	8 (0.0491)	11 (0.0675)	19 (0.1166)	144 (0.8834)	5 (0.0307)	4 (0.0245)	163	38 (0.2331)	163	22 (0.1350)
Sardinia	202	20 (0.0990)	1 (0.0050)	21 (0.1040)	181 (0.8960)	9 (0.0446)	0 (0.0000)	202	96 (0.4752)	202	7 (0.0347)
Sweden	360	23 (0.0639)	20 (0.0556)	43 (0.1194)	317 (0.8806)	10 (0.0278)	4 (0.01 11)	363	73 (0.2011)	363	91 (0.2507)
Denmark	261	15 (0.0575)	14 (0.0536)	29 (0.1111)	232 (0.8889)	6 (0.0230)	4 (0.0153)	261	62 (0.2375)	261	85 (0.3257)
Norway	469	37 (0.0789)	21 (0.0448)	58 (0.1237)	411 (0.8763)	18 (0.0384)	2 (0.0043)	469	125 (0.2665)	469	145 (0.3092)
Northern Ireland	150	7 (0.0467)	4 (0.0267)	11 (0.0733)	139 (0.9267)	3 (0.0200)	1 (0.0067)	150	39 (0.2600)	150	43 (0.2867)
England	1359	75 (0.0552)	53 (0.0390)	128 (0.0942)	1231 (0.9058)	41 (0.0302)	4 (0.0029)	441	142 (0.3220)	441	102 (0.2313)
The Netherlands	670	38 (0.0567)	32 (0.0478)	70 (0.1045)	600 (0.8955)	21 (0.0313)	2 (0.0030)	166	40 (0.2410)	670	168 (0.2507)
Czech Republic	100	2 (0.0200)	2 (0.0200)	4 (0.0400)	96 (0.9600)	2 (0.0200)	0 (0.0000)	100	23 (0.2300)	100	18 (0.1800)
Austria	637	17 (0.0267)	24 (0.0377)	41 (0.0644)	596 (0.9356)	8 (0.0126)	2 (0.0031)	637	159 (0.2496)	637	151 (0.2370)
Germany	4607	221 (0.0480)	102 (0.0221)	323 (0.0701)	4284 (0.9299)	60 (0.0130)	14 (0.0030)	4654	1017 (0.2185)	4607	649 (0.1409)
Spain	283	17 (0.0601)	19 (0.0671)	36 (0.1272)	247 (0.8728)	7 (0.0247)	3 (0.0106)	283	95 (0.3357)	283	50 (0.1767)
Belgium	329	18 (0.0547)	16 (0.0486)	34 (0.1033)	295 (0.8967)	4 (0.0122)	1 (0.0030)	329	90 (0.2736)	325	63 (0.1938)
Luxembourg	75	1 (0.0133)	1 (0.0133)	2 (0.0267)	73 (0.9733)	0 (0.0000)	0 (0.0000)	75	17 (0.2267)	75	16 (0.2133)
France	476	18 (0.0378)	16 (0.0336)	34 (0.0714)	442 (0.9286)	9 (0.0189)	2 (0.0042)	419	132 (0.3150)	476	79 (0.1660)
Portugal	181	13 (0.0718)	4 (0.0221)	17 (0.0939)	164 (0.9061)	6 (0.0331)	0 (0.0000)	181	58 (0.3204)	181	22 (0.1215)
Italy	375	4 (0.0107)	1 (0.0027)	5 (0.0133)	370 (0.98 67)	2 (0.0053)	0 (0.0000)	380	73 (0.1921)	263	21 (0.0798)
Greece	122	2 (0.0164)	4 (0.0328)	6 (0.0492)	116 (0.9508)	0 (0.0000)	0 (0.0000)	122	34 (0.2787)	122	6 (0.0492)
Israel	252	6 (0.0238)	5 (0.0198)	11 (0.0437)	241 (0.9563)	5 (0.0198)	1 (0.0040)	252	72 (0.2857)	252	12 (0.0476)
Poland	158	4 (0.0253)	1 (0.0063)	5 (0.0316)	153 (0.9684)	2 (0.0127)	0 (0.0000)	158	40 (0.2532)	158	34 (0.2152)
Hungary	62	1 (0.0161)	0 (0.0000)	1 (0.0161)	61 (0.9839)	0 (0.0000)	0 (0.0000)	62	8 (0.1290)	62	8 (0.1290)
Macedonia	103	5 (0.0485)	2 (0.0194)	7 (0.0680)	96 (0.9320)	0 (0.0000)	0 (0.0000)	103	19 (0.1845)	103	20 (0.1942)
Romania	67	4 (0.0597)	3 (0.0448)	7 (0.10 45)	60 (0.8955)	0 (0.0000)	0 (0.0000)	67	21 (0.3134)	67	6 (0.0896)
Bulgaria	154	2 (0.0130)	2 (0.0130)	4 (0.0260)	150 (0.9740)	1 (0.0065)	0 (0.0000)	154	33 (0.2143)	154	18 (0.1169)
Russia	121	3 (0.0248)	3 (0.0248)	6 (0.0496)	115 (0.9504)	1 (0.0083)	0 (0.0000)	121	24 (0.1983)	121	16 (0.1322)
Total	11736	561 (0.0478)	361 (0.0308)	922 (0.0786)	10814 (0.9214)	220 (0.0187)	44 (0.0037)	10312	2530 (0.2453)	10705	1852 (0.1730)

Table 3. Summary of correlation analysis: HLA-DQ genotype frequency compared with population incidence of Type I diabetes

HLA-DQ genotype class	HLA-DQ genotypes involved	Test of correlation	
		Correlation coefficient τ (SEM)	*p*
All (Strong + Weak)	*DQ2/DQ8; DQ2/DQ2; DQ8/DQ8; DQ4/DQ8; DQ5/DQ8; DQ6/DQ8*	0.437 (0.097)	< 0.001
Strong	*DQ2/DQ2; DQ2/DQ8; DQ8/DQ8*	0.413 (0.123)	< 0.001
Max	*DQ2/DQ8*	0.540 (0.099)	< 0.001
Strong except Max	*DQ2/DQ2; DQ8/DQ8*	0.307 (0.163)	0.060
Weak	*DQ4/DQ8; DQ5/DQ8; DQ6/DQ8*	0.337 (0.136	0.013
DQ4/DQ8	*DQ4/DQ8*	0.400 (0.139)	0.0040
Weak except DQ4/DQ8	*DQ5/DQ8; DQ6/DQ8*	0.241 (0.134)	0.072
Rest	All genotypes not included in "All"	–0.437 (0.097)	< 0.001
ASP-	All genotypes without haplotypes coding for Asp at pos. 57 of the DQβ-chain	0.200 (0.156)	0.20
DQ6/X	All genotypes that include DQB1*0602	0.287 (0.156)	0.066

The *DQ* genotypes were given a susceptibility score according to the formation of possible heterodimers, as suggested previously [9, 16]. We combined the six genotypes associated with Type I diabetes risk in Caucasians into one increased risk category classified as the genotype "All" in the calculations [19, 22]. Further separate analyses were performed for the category "Max" comprising the DQA1*0501-DQB1*0201/DQA1*0301-DQB1*0302 (DQ2/DQ8) genotype, as well as for the category "Strong" comprising the "Max" (DQA1*0501-DQB1*0201/DQA1*0301-DQB1*0302) genotype together with the two homozygous combinations of its haplotypic components, DQA1*0301-DQB1*0302/DQA1*0301-DQB1*0302 *(DQ8/DQ8)* and DQA1*0501-DQB1*0201 / DQA1*0501-DQB1*0201 *(DQ2/DQ2)*. The remaining genotypes in the "All" class are denoted "Weak", and include DQA1*0101-DQB1*0501/DQA1*0301-DQB1*0302 *(DQ5/DQ8)*, DQA1*0102-DQB1*0604/DQA1*0301-DQB1*0302 *(DQ6/DQ8)* and DQA1*0401-DQB1*0402/DQA1*0301-DQB1*0302 *(DQ4/DQ8)*. In addition, we analysed the distribution of DQB1 non-Asp57 homozygosity (named genotype "ASP-") and of the Type I diabetes protective haplotype DQA1*0102-DQB1*0602 (named genotype "*DQ6/X*").

The genotype data, for each country is shown in Table 2. Even though genetic marker data exists from Ireland, this nation has not been included in the analysis because of the scarcity of epidemiological information, leaving Northern Ireland to represent the whole island.

Statistical analysis. An initial analysis was done by calculating Kendall's τ (with adjustment for ties) [23] to examine the correlation between genotype frequencies and Type I diabetes incidence rates. This analysis weights each country equally and does not allow the relationship between genotype frequency and Type I diabetes incidence rate to be quantified. A straightforward regression of incidence rate on genotype frequency would not be correct because of the uncertainty in both variables [24, 25]. In our case the genotype frequencies and the Type I diabetes incidence rates are based on independent groups of individuals. This allowed us to develop a simple probability-based approach assuming binomial distribution of the *HLA-DQ* genotypes and standard Poisson distribution of the incident cases. This yields directly the joint probability function

$$L \propto \prod_{i=1}^{25} p_i^{X_i} (1-p_i)^{n_i - X_i} \lambda_i^{D_i} e^{-\lambda_i S_i}$$

with, for population i,
X_i = number of individuals with high risk genotypes
n_i = total number of individuals genotyped
p_i = population frequency of high risk genotype
D_i = number of new Type I diabetes patients observed
S_i = number of person-years observed
λ_i = population Type I diabetes incidence.

We hypothesised that if a linear association exists between Type I diabetes incidence and genotype frequency, which could be reduced to proportionality with no association and no incidence variation as the extreme. This hypothesis was formally tested by the probability ratio criterion, with the full model (free variation, hypothesis H_0) as reference for H_1, and H_1 as reference for the other models.

Results

The results of the correlation analysis are summarized in Table 3. For the group of genotypes included in the class "All" there was evidence of a positive correlation with the population incidence of Type I diabetes ($p < 0.001$). However, when analysing subgroups of genotypes this evidence was established by two distinct genotypes, the "Max" genotype (DQA1*0501-DQB1*0201/DQA1*0301-DQB1*0302) from the "Strong" subgroup of "All", and the "*DQ4/DQ8*" genotype (DQA1*0401-DQB1*0402/DQA1*0301-DQB1*0302) from the "Weak" subgroup of "All". The remaining subgroups of genotypes in "All" did not reach statistical significance in the correlation. Neither of the separate groups "ASP-" (DQB1-non Asp57 homozygozity) and "*DQ6/X*" (DQA1*0102-DQB1*0602 positive genotypes) showed a statistically significant correlation with the population Type I diabetes incidences. It is noteworthy that the protective genotype "*DQ6/X*" showed close to a significant *positive* correlation ($p = 0.066$). Plots of the correlation data are shown in Figure 1. The group "Rest", representing the complement of "All", mirrors the correlation pattern of "All".

In agreement with the initial correlation analysis, a linear model could only be fitted for the two genotypes "Max" (DQA1*0501-DQB1*0201/

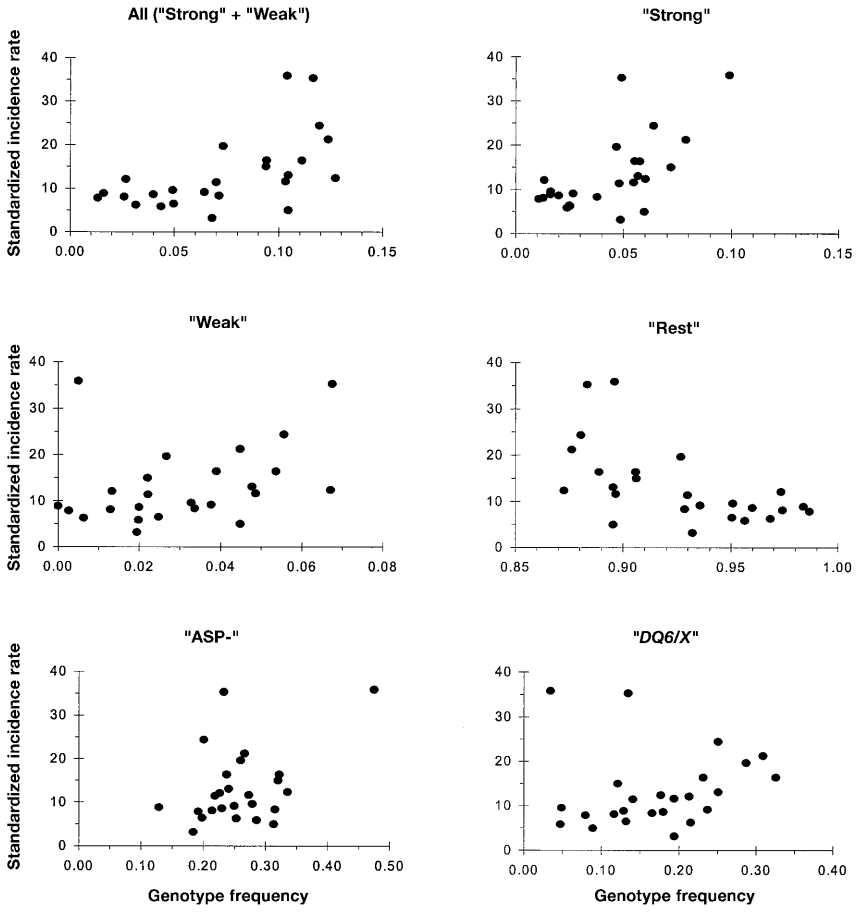

Fig. 1. Corresponding values of standardized incidence levels of Type I diabetes and population frequencies of HLA-DQ genotypes. Incidence rates are expressed per 100 000 person-years

DQA1*0301-DQB1*0302) and "*DQ4/DQ8*" (DQA1*0401-DQB1*0402/DQA1*0301-DQB1*0302). For both of these, the linear model could be further reduced to proportionality with regression coefficients (and SEM) for "Max" and "*DQ4/DQ8*" at 700 (47) and 3500 (530), respectively and corresponding p values of 0.89 and 0.57, respectively. The fitted models are shown in Figure 2. The probability based fit takes account of both the uncertainty in ordinate (diabetes incidence), as in ordinary regression, and the uncertainty in the abscissa (genotype frequency). This explains why the fit through the origin is marginally poorer than a fit with a freely varying intercept.

Both Finland and Sardinia represent outliners, with extremely high incidence rates and special characteristics of their population's genetic history and composition. However, excluding Finland and Sardinia from the analysis hardly changed the results (data not shown).

Discussion

An ecological analysis like ours could give rise to bias and limitations in interpretation [26]. Such biases are less relevant in the present context because we are dealing with genetic factors that are supposed to remain stable throughout life. It is, however, important to emphasise that our analysis describes variation between populations as a whole and cannot be immediately applied to determine individual susceptibility. A new statistical approach has been used to correct formally for variability in the sizes of the samples that provide incidence information as well as genetic marker information. This approach can be used to refine ecological analyses in general.

Our study has shown that the geographical variability of the population risk of Type I diabetes in Europe is partly attributable to the geographical variability in the population frequency of two distinct *HLA-DQ* susceptibility genotypes, namely *DQ2/DQ8* (DQA1*0501-DQB1*0201/DQA1*0301-DQB1*0302) and *DQ4/DQ8* (DQA1*0401-DQB1*0402/DQA1*0301-DQB1*0302). In line with this finding, the frequencies of these genotypes are highest among Type I diabetic patients in the northern part of Europe and lowest in southern Europe [19, 27].

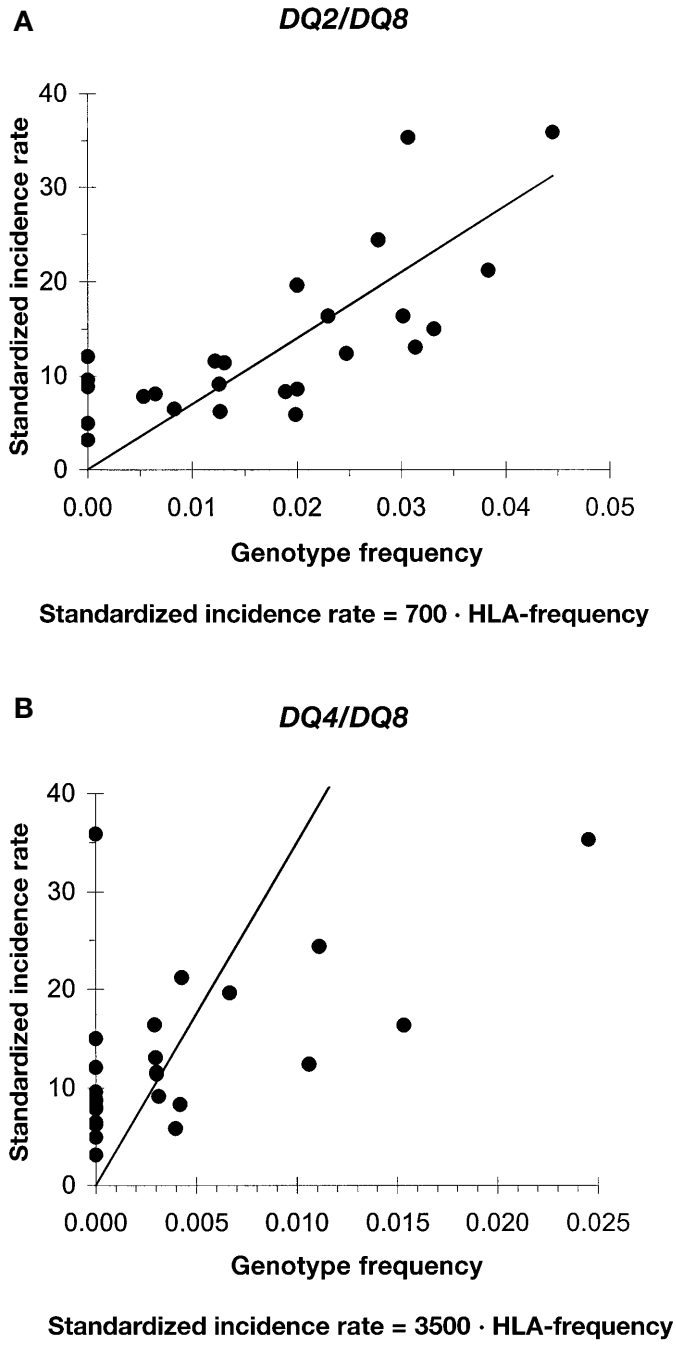

Fig. 2 A, B. Corresponding values of standardized incidence levels of Type I diabetes and population frequencies of the HLA genotypes *DQ2/DQ8* (**A**) and *DQ4/DQ8* (**B**), respectively. Lines indicate fit to proportionality model (Table 3). Incidence rates are expressed per 100 000 person-years

The HLA associations for Type I diabetes show heterogeneity within the European Caucasian population. While different genotypic combinations with the *DR4*-DQA1*0301-DQB1*0302 haplotype are most important in northern Europe, different combinations with the *DR3*-DQA1*0501-DQB1*0201 haplotype are more important in southern Europe [19, 27]. The same is the case for the homozygous combinations of these two haplotypes because *DR3*-DQA1*0501-DQB1*0201 homozygosity plays the same role in southern Europe as *DR4*-DQA1*0301-DQB1*0302 does in northern Europe [19, 27]. Based on more recent knowledge of the different contribution of *HLA-DQ* genotypes within Europe, the correlation between incidence and genomic markers can only be determined when we know which genotypes confer Type I diabetes risk in a given population. Such data are also of enormous importance for Type I diabetes prediction and intervention studies.

We are well aware of the difficulties involved in defining a population on the principle of the nation state and therefore undertooked a separate analysis of Northern Ireland and Sardinia. The vast majority of the countries in our analysis are, however, homogenous. The changes in the political map of Europe over the past decade have brought a closer identification between state and nation. The Baltic states have separated from Russia, the Czechs from Slovaks. There are two states in our analysis to which this rule might not apply: these are two successor states of the former Yugoslavia, Croatia and the Republic of Macedonia.

In contrast to previous studies which attributed much of the geographic differences in Type I diabetes incidence to variations in the prevalence of the *DQB1* non-Asp57 alleles in a population, we did not find that the prevalence of *DQB1* Asp57 negative genotypes (referred to as "ASP-") was correlated with Type I diabetes incidence ($p = 0.20$) [28, 29]. Our results confirm previous findings [30] presenting a more complex view of the genetics of Type I diabetes in a population. Our study has also not been able to demonstrate any protective effect of the HLA-DQB1*0602 allele at the population level. Conversely, its prevalence in the population was a close to significant positive correlation to Type I diabetes incidence ($p = 0.066$).

The presence of a strong positive correlation between population frequency of genetic susceptibility markers and population disease risk does not in itself exclude the importance of non-genetic factors (or genetic factors not accounted for in the analysis). All populations currently considered to be at a high and even extremely high Type I diabetes risk (eg. in Finland and the in other Nordic countries; Sardinia; UK), have had dramatic increases in the Type I diabetes incidence over the past decades, compared to a slower increase or no increase earlier [1, 31–33]. The increases cannot be explained by secular changes in the pool of susceptibility genes and imply that no correlation would have been detected between population frequency of susceptibility genotypes and the population risk of Type I diabetes if our analysis had been performed a few decades ago. These findings suggest that non-genetic causes interact with the genetic susceptibility factors in Type I diabetes and

that such an interaction has been enhanced over the past decades in subjects carrying specific high-risk genotypes. If so, the distribution of HLA-genotypes in newly diagnosed Type I patients should change over time in populations who have changing incidences of Type I diabetes. The hypothesis could be supported by the rapid increase of Type I diabetes incidence in children in the age group 0–4 years, observed in particular in high-incidence populations of Finland and the UK [34–36]. This is because the prevalence of the risk genotypes is generally highest in the diabetic children with early disease onset [27]. Interaction between specific high-risk genotypes and early exposure to environmental risk factors (e.g. enteroviral infections) is believed to trigger beta-cell autoimmunity in susceptible individuals, because children converting to islet cell antibodies (ICA) positivity following enterovirus infection have been shown to carry high-risk *HLA-DQB1* genotypes more often than children who remained ICA negative [37].

Our study indicates avenues for further research to explain the complex causes of Type I diabetes. The currently defined high-risk populations are excellent for studies of gene-environment interactions in causing of Type I diabetes. However, comparative studies in low-risk populations could serve as an important reference. This type of research must necessarily incorporate genotyping of patients with Type I diabetes from the same populations.

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