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DIABETES

Incidence rate of type 1 diabetes in Santiago (Chile) by HLA-DQA1 and DQB1 genotypes

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Abstract. The aim of this study was to estimate annual incidence rate of type 1 diabetes according to the levels of genetic susceptibility provided by HLA-DQA1 and HLA-DQB1 genotypes. Two information sources were used: (1) a population-based incidence study in which 61 incident cases were ascertained during 1 year in Santiago, Chile (incidence rate: 4.11 cases per 100,000 children per year) and (2) a case–control comparison of 57 cases (recruited from the incidence study) and 125 controls. Susceptibility alleles were defined as DQA1*0301 and DQA1*0501 for DQA1 gene and alleles DQB1*0201 and DQB1*0302 for DQB1 gene. In DQA1 gene, the highest point estimate of the incidence rate was calculated for the genotype DQA1*0501/DQA1*0501 (33.04 cases per 100,000 children aged less than 15 years old and per year; 95% CI: 9.22–118.33). In the DQB1 gene, the highest risk was estimated for the genotype DQB1*0201/DQB1*0201 (20.35 cases per 100,000 children aged less than 15 years old and per year; 95% CI: 5.26–78.67). This study shows an application on how a transformation of the logistic equation based on Bayes' theorem can be used to estimate incidence rates from case–control studies and population-based incidence rates.

Key words: Diabetes, HLA-DQ genotypes, Incidence

Introduction

Type 1 diabetes is a complex disorder characterised by an autoimmune destruction of pancreatic β cells producing insulin [1]. A number of ecological studies have tested the hypothesis that the remarkable geographic differences in the incidence of this disease are partly explained by the disparity in the frequency of susceptibility alleles of the human leukocyte antigen (HLA) system [2–4]. The incidence rate of type 1 diabetes in the Metropolitan Region of Santiago, Chile (urban and rural areas) has been increasing during the last years although with figures still lower than most of the European countries [5].

Prediction of type 1 diabetes either in relatives of cases or in the general population, based on genetic and/or immune markers, is a recurrent topic in the scientific literature [6–8]. Population-based follow-up studies such as DIPP and DAISY projects [8, 9] have managed different screening approaches with the ultimate goal of predicting and eventually preventing the onset of the disease in susceptible subjects.

The purpose of this study is to estimate the annual incidence rate (one-year risk) of type 1 diabetes in Chilean population by levels of genetic susceptibility defined by HLA-DQA 1 and HLA-DQB1 genotypes making use of the information derived from an earlier population-based incidence study and HLA-disease associations estimated from case–control comparisons.

Subjects

A total of 61 type 1 diabetes cases aged less than 15 years at onset were ascertained in the urban area of Santiago, Chile from March 21, 1997 to March 20, 1998. These cases were ascertained as part of the activities leading to the establishment of the population-based registry of type 1 diabetes in the Metropolitan Region of Santiago and represent all the cases diagnosed in the study period. According to post-censal projections, the population 'at-risk' (< 15 years of age) at the beginning of the study period was 1,483,218 subjects (Instituto Nacional de Estadísticas, 1993). The incidence rate of that year was calculated as 4.11 cases per 100,000 children age less than 15 years per year [10].

Among the whole set of 61 affected subjects ascertained during the incidence study (March 21, 1997 to March 20, 1998), 57 cases were genotyped for HLA-DQ genes in published family study [11]. On the other hand, 125 healthy unrelated children constituted the control group from a previous case–control study [12, 13]. An explanation of the subjects recruitment process (cases and controls) has been previously described [10–

13]. The proportion of ICA positive cases ($\geqslant$20 Juvenile Diabetes Foundation Units) was 0.73 [14]. The present study has been thought as a re-analysis of published data combining HLA-DQ genotypes from unaffected subjects [12] and genotypes from incident cases diagnosed during the study period [10, 11] combined in a new case–control analysis.

Laboratory methods

Cases and controls were genotyped using polymerase chain reaction and dot-blot hybridization with oligoprobes distinguishing five groups of DQA1 alleles and seven groups of DQB1 alleles as described previously [11, 12]. The groups of alleles identified were DQA1*0101, DQA1*0201, DQA1*0301, DQA1*-0401 and DQA1*0501 in DQA1 locus and DQB1*201, DQB1*301, DQB1*302, DQB1*303, DQB1*402, DQB1*501 and DQB1*602 in DQB1 locus. Four DNA samples were not successfully assayed for DQB1 gene.

Susceptibility alleles have been defined in this study as those reported to be part of the main diabetogenic HLA-DQ haplotypes for most populations (DQA1*0301 and DQA1*0501 alleles for DQA1 gene and DQB1*0201 and DQB1*0302 alleles for DQB1 gene) [15]. Several classification schemes are possible to define low-risk or high-risk genotypes. Non-susceptibility alleles are collectively grouped in a category called allele N (where N stands for neutrality or relative protection). Likewise, susceptibility alleles themselves may be grouped in a category called allele S (where S stands for susceptibility). According to the latter simplified classification scheme, a person may carry zero, one or two copies of susceptibility alleles either in HLA-DQA1 or HLA-DQB1 genes (genotypes NN, NS and SS respectively).

Statistical methods

We estimated the incidence rate of type 1 diabetes by HLA-DQA1 and HLA-DQB1 genotypes using a modification of the intercept of the logistic regression equation. A logistic model with binary variables for DQA1 or DQB1 genotypes was employed. The binary variable that indicates the NN genotype was not included in the models and served as reference.

The procedure for calculating incidence rates was as follows. First, we transformed the intercept of the regression logistic model (see Appendix 1) using the incidence rate for Santiago (4.11 cases per 100,000 children age less than 15 years per year). Second, we replaced the new intercept in the logistic equation to calculate incidence rate figures for groups of individuals carrying different genotypes. This statistical procedure is based on the use of Bayes' theorem in the logistic model. Finally, we calculated standard errors and confidence intervals for such estimates [16].

Results

Tables 1 and 2 show DQA1 and DQB1 genotypes in cases and controls, the odds ratios and the calculated incidence rates for the different genotypes with their corresponding 95% confidence intervals. The estimation of incidence rates in Tables 1 and 2 are quite unstable due to the small sample size relative to the number of genotypes involved in the calculation. For this reason, we have also used a simplified genotype classification for genotypes with zero, one or two copies of susceptibility alleles (Tables 3 and 4).

Discussion

The overall incidence rate of type 1 diabetes represents a weighed average of incidence rates of different subgroups of subjects characterised by their genetic susceptibility, which is mainly determined by their HLA genetic profile [2–4]. This study shows an application on how the logistic model is useful not only to estimate risk in follow-up studies, but also to estimate incidence rates using case–control studies combined with population-based incidence rates,

Table 1. Incidence rate of type 1 diabetes in Santiago (Chile) by HLA-DQA1 genotypes

HLA-DQA1 genotypes	Controls	Cases	Odds ratio (95% CI)	Incidence rate* (95% CI)
N/N	44 (35.20%)	4 (7.02%)	–	0.82 (0.29–2.28)
301/N	29 (23.20%)	11 (19.30%)	4.17 (1.21–14.37)	3.42 (1.71–6.84)
501/N	26 (20.80%)	2 (3.51%)	0.85 (0.15–4.94)	0.69 (0.16–2.92)
301/501	10 (8.00%)	21 (36.84%)	23.10 (6.48–82.32)	18.92 (8.91–40.18)
301/301	13 (10.40%)	8 (14.04%)	6.77 (1.75–26.12)	5.55 (2.30–13.38)
501/501	3 (2.40%)	11 (19.30%)	40.33 (7.85–207.15)	33.04 (9.22–118.33)
Total	125	57		

Allele N are those alleles different than the DQA1 susceptibility alleles (DQA1*0301 and DQA1*0501 are the susceptibility alleles in DQA1 gene).

* Incidence rate is calculated as number of new cases per 100,000 inhabitants aged less than 15 years of age per year.

Table 2. Incidence rate of type 1 diabetes in Santiago (Chile) by HLA-DQB1 genotypes

HLA-DQB1 genotypes	Controls	Cases	Odds ratio (95% CI)	Incidence rate* (95% CI)
N/N	52 (42.98%)	3 (5.26%)	–	0.5 (0.16–1.61)
201/N	20 (16.53%)	7 (12.28%)	6.07 (1.43–25.79)	3.05 (1.29–7.22)
302/N	31 (25.62%)	16 (28.07%)	8.94 (2.41–33.17)	4.50 (2.46–8.23)
201/302	4 (3.31%)	6 (10.53%)	25.99 (4.66–145.00)	13.09 (3.69–46.36)
201/201	3 (2.48%)	7 (12.28%)	40.43 (6.79–240.77)	20.35 (5.26–78.67)
302/302	11 (9.09%)	18 (31.58%)	28.36 (7.10–113.21)	14.28 (6.74–30.22)
Total	121	57		

Allele N are those alleles different than susceptibility alleles in DQB1 locus (DQB1*0201 and DQB1*0302 are the susceptibility alleles in DQB1 gene).
* Incidence rate is calculated as number of new cases per 100,000 inhabitants aged less than 15 years of age per year.

Table 3. Incidence rate of type 1 diabetes in Santiago (Chile) by HLA-DQA1 genotypes in a simplified scheme (NN, NS and SS genotypes)

HLA-DQA1 susceptibility alleles	Controls	Cases	Odds ratio (95% CI)	Incidence rate* (95% CI)
No susceptibility alleles (NN genotype)	44 (35.20%)	4 (7.02%)	–	0.82 (0.29–2.28)
One copy of susceptibility Alleles (NS genotype)	55 (44.00%)	13 (22.81%)	2.60 (0.79–8.54)	2.13 (1.16–3.90)
Two copies of susceptibility Alleles (SS genotype)	26 (20.80%)	40 (70.18%)	16.92 (5.43–52.73)	13.87 (8.46–22.72)
Total	125	57		

Susceptibility alleles (or S alleles) are DQA1*0301, DQA1*0501. Other HLA-DQA1 alleles are grouped as allele N (conferring neutrality or relative protection).
* Incidence rate is calculated as number of new cases per 100,000 inhabitants aged less than 15 years of age per year.

Table 4. Incidence rate of type 1 diabetes in Santiago (Chile) by HLA-DQB1 genotypes in a simplified scheme (NN, NS and SS genotypes)

HLA-DQB1 susceptibility alleles	Controls	Cases	Odds ratio (95% CI)	Incidence rate* (95% CI)
No susceptibility alleles (NN genotype)	52 (42.98%)	3 (5.26%)	–	0.50 (0.16–1.61)
One copy of susceptibility Alleles (NS genotype)	51 (42.15%)	23 (40.35%)	7.82 (2.21–27.65)	3.93 (2.41–6.44)
Two copies of susceptibility Alleles (SS genotype)	18 (14.88%)	31 (54.39%)	29.84 (8.13–109.56)	15.02 (18.41–26.85)
Total	121	57		

Susceptibility alleles (or S allele) are DQB1*0201 and DQB1*0302. Other HLA-DQB1 alleles are grouped as allele N (conferring neutrality or relative protection).
* Incidence rate is calculated as number of new cases per 100,000 inhabitants aged less than 15 years of age per year.

through an appropriate transformation of the logistic equation intercept.

The overall incidence rate calculated for the study period (4.11/100,000 children per year) was managed as a parameter and not as an estimate, and therefore there is no standard error associated with such figure. This approach is based on the fact that all cases were ascertained in the whole population during March 21,

1997 to March 20, 1998. Consequently, the incidence rates for the different levels of genetic susceptibility shown in Tables 1 and 2 would only strictly apply for that year and could not be extended to future predictions. However, such estimates could serve as an approximate guidance for incidence rates and their confidence intervals for the different levels of genetic susceptibility defined by HLA genotypes in a more general context. On the other hand, risk estimates reported in this study are equivalent to the ones calculated through the use of Bayes' theorem [4, 17].

The term 'Allele S' combines different HLA-DQ genetic variants with different degrees of susceptibility to type 1 diabetes. Moreover, the effect of HLA-DQ haplotypes is also modulated by alleles in the DRB1 gene or environmental risk factors [18]. For all these reasons, incidence rates by HLA-DQ genotypes constitute only average figures that do not take into account the complexity of the disease causation process. Likewise, allele N also represents a group of alleles with different degree of relative protection against the disease. For example, it is accepted that DQB1*0602 allele has a protective effect for the disease [19]. On the other hand, DQA1 and DQB1 genes are in strong linkage disequilibrium. Because of this effect, DQA1 genotypes could be inferred from the DQB1 genotype in many situations. However, this prediction is population-dependent and not always accurate [19], especially in mixed populations such as the Chilean population [20]. For this reason, we have computed incidence rates separately for DQA1 and DQB1 genes. We did not address the differential HLA pattern by age-at-onset in type1 diabetes and consequently the incidence rates. The incidence rates estimates by HLA genetic profile only constitute average figures computed from cases diagnosed at different ages.

The Chilean population constitutes a melting pot of populations arising from different parts of the world including Europeans of different origins, mainly Spain, and Amerindians [20, 21]. The incidence rate of the disease is low compared to European countries [5, 22]. The frequency of Chilean patients diagnosed from March 21, 1997 to March 20, 1998 in Santiago who are positive for DQB1*0201 and DQB1*0302 alleles were 0.35 and 0.7 respectively. Therefore, the most frequent allele found in Chilean patients is DQB1*0302 and the same general situation seems to occur in Finnish type 1 diabetes cases but not in Greek patients [19]. In contrast, the frequency of controls positive for DQB1*0302 allele would be higher in Chile (0.38) than in Finland or Greece [19]. We have compared carrier frequencies of the main DQB1 susceptibility alleles of Chilean patients with the ones published for Finland and Greece [19, 22]. These countries have very high and very low incidence of the disease in the European context and show different degrees in the strength of association for specific HLA-DQ genotypes. On the other hand, no Chilean patients diagnosed during the study period in Santiago were positive for the DQB1*0602 allele while 17% of controls were positive for such allele.

The positive predictive values for the disease according to HLA-DQA1 and DQB1 genotypes are low in spite of the strong effect of some HLA alleles on disease risk. The pre-test risk of developing the disease is low in Chilean population which limits the use of HLA loci as useful post-test predictors, even considering the strong effect of HLA genotypes on disease risk. Specifically, we have estimated that the 15-year risk for a random Chilean newborn to develop type 1 diabetes is around 0.06% while the 15-year risk for a Chilean newborn who carry the SS genotype in DQB1 gene is around 0.23%. Positive predictive values for type 1 diabetes provided by HLA-DQ genotypes are in general higher than the predictive values calculated for other single genetic markers in most common disorders, except for highly penetrant genetic variants. In this context, some authors have visualised genetic testing as a tool for predicting and preventing the occurrence of complex diseases by the avoidance of environmental factors that interact with specific genetic factors [23]. However, a public health perspective has shown the difficulty in using such a strategy [24]. Cost of the screening programs and availability of adequate preventive measures are also important issues to consider in population genetic testing [7, 19]. On the other hand, although no satisfactory preventive action is available up to now for type 1 diabetes, prevention trials in humans and interventions in animal models are in current development and may establish future clinical interventions to prevent the disease in susceptible individuals [25].

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Appendix 1

Let us write the logistic equation in a cohort study as

$$\text{logit} = \log(p/(1-p)) = \alpha_{CO} + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_n x_n \qquad (1)$$

where α_{CO} is the intercept and the β's are the coefficients for the regression model. In a case–control study, the logistic model continues to apply with the same coefficients β's but a different intercept denoted by α_{CC}. Using Bayes' theorem, the relation between α_{CC} and α_{CO} [16] would be expressed as

$$\alpha_{CO} = \alpha_{CC} - \log(\pi_0/\pi_1) \qquad (2)$$

where π_0 is the sampling proportion for cases and π_1 is the sampling proportion for non-diseased subjects.

Let us consider an 'at-risk' population of size N in which all incident cases are ascertained during a complete year. Let us consider also a case–control study embedded within such a population-based incidence study. Let us define the following terms: A is the total number of cases occurred in the population during a year; B, the total number of subjects at risk who remained disease-free after a year.

$$N = A + B \tag{3}$$

a is the number of cases (among A diseased subjects) selected in the case–control study; b, the number of controls (among B diseased-free subjects) selected in the case–control study.

Sampling proportions π_0 and π_1 are defined as

$$\pi_0 = Pr(Z = 1/D) \quad \text{and} \quad \pi_1 = Pr(Z = 1/\overline{D})$$

where D denotes the cases and $\overline{D}$ denotes the non-cases. The binary variable Z indicates whether a subject is selected or not for the case–control study.

By replacing a, A, b and B in the Equation (2), we obtain

$$\begin{aligned}\alpha_{CO} &= \alpha_{CC} - \log(\pi_0/\pi_1) = \alpha_{CC} - \log[(a/A)/(b/B)] \\ &= \alpha_{CC} - \log[(a/b)/(A/B)]; \\ \alpha_{CO} &= \alpha_{CC} - \left[\log\left(\frac{a}{b}\right) - \log\left(\frac{A}{B}\right)\right]\end{aligned} \tag{4}$$

Let us denote π as the incidence rate of the disease during a year.

$\pi = \dfrac{A}{N}$ replacing N according to (3):

$$\pi = \frac{A}{A+B} \quad \text{and} \quad \frac{\pi}{1-\pi} = \frac{A}{B}$$

By replacing these terms in (4), we get

$$\begin{aligned}\alpha_{CO} &= \alpha_{CC} - \left[\log\left(\frac{a}{b}\right) - \log\left(\frac{\pi}{1-\pi}\right)\right]; \\ \alpha_{CO} &= \alpha_{CC} - \log\left(\frac{a}{b}\right) + \log\left(\frac{\pi}{1-\pi}\right) \\ \text{logit} &= \log(p/(1-p)) = \alpha_{CO} + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_n x_n\end{aligned}$$

Let us denote $\text{LCL}_{\text{logit}}$ and $\text{UCL}_{\text{logit}}$ as the 95% lower confidence limit and the 95% upper confidence limit for logit, respectively:

$$\text{LCL}_{\text{logit}} = \text{logit} - 1.96 * \text{se.} \quad \text{and} \quad \text{UCL}_{\text{logit}} = \text{logit} + 1.96 * \text{se.},$$

where se. is the covariance matrix of $\beta = (\alpha_{CC}, \beta_1, \beta_2, \ldots, \beta_n)^T$ (π is considered as a parameter).

Let us denote LCL_p and UCL_p as the 95% lower confidence limit and the 95% upper confidence limit for p, respectively:

$$\text{LCL}_p = 1/(1 + \exp(-\text{LCL}_{\text{logit}})) \quad \text{and}$$
$$\text{UCL}_p = 1/(1 + \exp(-\text{UCL}_{\text{logit}}))$$

This expression would permit to get the intercept that would have been obtained in a cohort study from (a) the intercept of a case–control study, (b) the number of cases and the controls, and (c) the incidence rate of the disease. The replacement of this expression in Equation (1) enables the computation of the probability p by the different levels of the predictor variables. The conversion of the intercept and the interval estimation of p have been implemented in an *S-PLUS* program freely available from the first author (noradiaz@uchile.cl).

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