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RESEARCH PAPER

Type 1 Diabetes in the Offspring does not Increase the Risk of Parental Type 2 Diabetes in South Indians

A. Ramachandran[1]*
C. Snehalatha[1]
E. Tuomilehto-Wolf[2]
G. Vidgren[2]
B.W. Ogunkolade[3]
V. Vijay[1]
G.A. Hitman[4]

[1]*Diabetes Research Centre, Madras, India*

[2]*National Public Health Institute, Helsinki, Finland*

[3]*Medical Unit, St Bartholomew's, London, UK*

[4]*The Royal London School of Medicine and Dentistry, London, UK*

*Correspondence to: Diabetes Research Centre, 5 Main Road, Royapuram, Madras 600 013, India.
E-mail: ramachandran@vsnl.com

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Abstract

Objectives (a) To study whether there was an increased prevalence of glucose intolerance in the parents of probands with Type 1 diabetes and (b) to look for any possible link between the glucose intolerance in the parents with HLA-DQB1 alleles transmitted in excess to the Type 1 diabetes offspring.

Study Design and Methods From 215 families of South Indian Type 1 diabetes probands, 336 parents (170 fathers, age 30–70 years; 166 mothers, age 23–72 years) were studied by oral glucose tolerance test (GTT). Glucose intolerance in the parents was compared with the population data available. HLA-DQB1 alleles in 170 of the families were studied by the Olerup method (based on sequence specific primers) and the transmission disequilibrium test (TDT) was used to determine the Type 1 diabetes-associated DQB1 alleles.

Results Among the parents 11.2% had Type 2 diabetes which was similar to the population data of 11.6%. However there was a male predominence among the diabetic parents ($\chi^2 = 7.0$, $p = 0.008$), while in the population there was a female predominence. Prevalence of IGT was significantly more among the parents (13.6%) compared with the population data (9.1%) ($\chi^2 = 6.43$, $p = 0.011$). Both HLA-DQB1*0201 ($p < 0.0001$) and DQB1*0302 ($p = 0.0001$) were positively associated with Type 1 diabetes in the probands although 21% of the probands possessed neither DQB1*0201 or DQB1*0302. The distribution of glucose tolerance categories in the parents of the probands differed according to the presence of DQB1*0302 ($p = 0.035$) whilst no such differences existed for DQB1*0201.

Conclusions In summary, the presence of Type 1 diabetes in the South Indian offspring does not predict a higher occurrence of Type 2 diabetes in the parents. However, there is an increased occurrence of impaired glucose tolerance (IGT) among the parents. Family based studies demonstrate increased transmission of HLA-DQB1*0201 and HLA-DQB1*0302 with Type 1 diabetes similar to North American and European Caucasian subjects. Furthermore, HLA-DQB1*0302 may be a minor determinant of glucose tolerance in parents of offspring with Type 1 diabetes.

Keywords Type 1 diabetes; Type 2 diabetes; Asian Indians; familial predisposition; HLA DQB; parental diabetes

Introduction

Type 1 diabetes shows a strong genetic association with the HLA complex in all ethnic populations, although the susceptibility haplotypes are varied [1–3]. Type 2 diabetes is likely to be a heterogeneous disease which might have an overlap with Type 1 diabetes with respect to pathogenesis. A few

earlier studies in families of patients with Type 1 diabetes have shown evidence for such associations but none have systematically determined glucose tolerance in the parents [4–6]. Dahlquist *et al*. from Sweden noted that the risk of Type 1 diabetes in children increased when their relatives had Type 2 diabetes [5]. Chern *et al*. had also reported increased risk of Type 1 diabetes in the siblings of Type 1 diabetes probands in families with a Type 2 diabetes parent [6]. Similar observations were also recorded by Wagener *et al*. from Pittsburgh, USA [4]. In elderly men, Tuomilehto-Wolf *et al*. showed that the Finnish population had a common HLA predisposition for both Type 1 diabetes and Type 2 diabetes [7]. A link between the two forms of diabetes has been demonstrated by Rich *et al*. in the Wadena city health study from USA [8]. Some of the links observed between Type 1 and Type 2 diabetes in the above studies, could have occurred due to possible misclassification, considering the difficulties in clear-cut classification in a few cases. South Indians have a high prevalence of Type 2 diabetes [9,10]. Though it was considered that South Indians had a low prevalence of Type 1 diabetes [11], a recent epidemiological study showed that the incidence of Type 1 diabetes in Indians was increasing and was comparable to some European populations [12]. This study was undertaken in South Indian families of Type 1 diabetes probands to investigate: (a) whether there would be a higher prevalence of abnormal glucose tolerance (IGT and Type 2 diabetes) in the parents; and (b) to use a family based association method to investigate the association between HLA-DQB1 and Type 1 diabetes and to look for a correlation between glucose tolerance in the parents and transmitted HLA-DQB1alleles.

Subjects and methods

Patients and families

Type 1 diabetes probands in this study were attending the MV Hospital for Diabetes, and Diabetes Research Centre, Madras, India. All of them satisfied the WHO criteria for Type 1 diabetes [1], i.e. they had an acute onset of the disease, they had ketonuria or ketosis at presentation and required insulin for maintenance of life. In none of the probands selected for this study was there an ambiguity regarding the diagnosis of Type 1 diabetes. Probands were in the age range of 1–42 years. From 215 families of Type 1 diabetes probands, 336 parents (170 fathers, age 30–70 years; 166 mothers, age 23–72 years) were available for the study. The clinical details of the probands and parents are presented in Table 1. Classification of diabetes and IGT was done using the WHO criteria [1]. Glucose tolerance in the parents were tested by oral GTT (75 g glucose). Diagnosis of diabetes was ascertained in known cases from the clinical history and details of treatment. Blood samples were collected for HLA-DQB1 analyses in the majority of families. Plasma glucose was estimated within 30 min of blood collection by the glucose oxidase method, using Boehringer Mannheim reagents and a Hitachi 704 autoanalyser. Blood samples for HLA (EDTA blood) were stored at -70°C until analysis.

Table 1. Characteristics of the Type 1 diabetes probands and their tested parents. Values are mean $\pm$ SD

	Probands	
	Male $n=101$	Female $n=144$
Age (years)	18 ± 8.5	16 ± 7.6
Age at diagnosis (years)	15 ± 7.8	13 ± 6.7
Range (years)	(1–42)	(1–34)
BMI (kg/m^2)	18.4 ± 3.8	18.8 ± 4.6
Duration of Type 1 diabetes (years)	6 ± 5.0	5 ± 3.8
Range (years)	(1–20)	(1–15)
	Parents	
	Fathers $n=170$	Mothers $n=166$
Age (years)	45 ± 8.0	39 ± 8.6
BMI (kg/m^2)	25 ± 3.9	25.5 ± 4.8
Age at diagnosis of glucose intolerance (years)		
IGT	45 ± 9.9 ($n=25$)	40 ± 8.8 ($n=30$)
NIDDM	44 ± 8.3 ($n=32$)	42.9 ± 10.0 ($n=16$)
IDDM	38 ± 16.3 ($n=4$)	—

HLA-DQB1

DNA was extracted from the blood samples using a Flowgen kit (Gentra System Incorporation, Minneapolis, USA) from 170 families consisting of parents and the Type 1 diabetes proband.

HLA class II DQB1 genes were determined by the Olerup method [13]. DNA was amplified using the polymerase chain reaction (PCR) with sequence-specific-primers (SSP) and PCR products analysed in 2% agarose gels. DQB1 loci were amplified using low resolution kits (Dynal, Oslo, Norway) followed by eight PCR reactions to identify DQ2 to DQ9. Alleles were assigned as recognized by the 1995 HLA Nomenclature committee [14]. HLA-DQB alleles were analysed in (a) probands and (b) parents. Since we did not subtype all DQB1*04, *05 and 06* alleles subtypings were pooled and designated DQB1*040X, DQB1*050X and DQB1*060X, respectively.

Statistical analysis

Patient characteristics were compared by the *t*-test. Prevalence of diabetes and IGT in parents were age-adjusted to the population of South India (1991 census) by direct standardization method. Statistical package SPSS version 4.0.1 was used for the analysis. The extended transmission disequilibrium test (ETDT) [15] was used to identify alleles transmitted in excess from the parents to the Type 1 diabetes offspring. Those alleles significantly associated with Type 1 diabetes were then analysed in the parents for associations with glucose tolerance (normal, IGT, diabetes) using the χ^2-test.

Results

Glucose tolerance in parents

A total of 336 parents (fathers 170 and mothers 166) from 215 families with Type 1 diabetes proband were studied. Four fathers were known cases of Type 1 diabetes (2.3%). The clinical characteristics of the probands and the parents are shown in Table 1. Prevalences of diabetes and IGT, age-adjusted to the urban population of Tamil Nadu (1991 census) were 11.2% and 13.6% respectively. (Table 2) The corresponding prevalence in a population survey conducted in 1995 were 11.6% for diabetes and 9.1% for IGT. The prevalences of diabetes in fathers was significantly higher (12%) in comparison with the mothers (10.4%) ($\chi^2=7.0$, $p=0.008$). There was a higher occurrence of IGT among the parents (13.6% vs 9.1%), $\chi^2=6.43$, $p=0.011$) (Table 2). The prevalences of IGT were 20% in fathers and 15.1% in mothers ($\chi^2=1.1$, $p=0.3$).

Mothers were younger (age 39.0 ± 8.6 years) than the fathers (45 ± 8.0 years). The prevalence of diabetes in mothers when adjusted to the age distribution of fathers was 14.1%. Mean age at diagnosis of Type 2 diabetes in fathers and mothers were similar. In 18 fathers and seven mothers, Type 2 diabetes, was diagnosed earlier (mean duration 8.1 + 5.0 years; range 1–20 years). Four fathers had Type 1 diabetes (duration 15 + 5.4 years). All except two of the Type 2 diabetes parents were being treated with oral hypoglycaemic agents. Two patients who were being treated with insulin were also responding to oral hypoglycaemic agents for a minimum period of 8 years. The mean HbA1 in the known Type 2 diabetes patients was 9.0 ± 1.3 % (normal = 5–8%).

Sixteen male Type 1 diabetes probands and 19 female probands had diabetic fathers and eight male and eight female probands had diabetic mothers showing no specific sex linked inheritance of diabetes.

Table 2. Age-adjusted prevalence (%) of glucose intolerance in tested parents

	Diabetes		IGT	
	Parents $n=336$	Population data $n=2183$	Parents	Population data
M : F	170 : 166	1081 : 1102		
Men	12.0	10.4	20.0	8.8
Women	10.4[a]	12.7	15.1	9.5
M : W ratio	1.15	0.82	1.32	0.93
Total	11.2	11.6	13.6[b]	9.1

[a] Between fathers and mothers $\chi^2=7.0$, $p=0.008$.
[b] Parents vs population data $\chi^2=6.43$, $p=0.011$.

HLA in the families

The clinical details for the subset of Type 1 diabetes probands ($n=167$) studied for HLA-DQ were mean age of onset, 14 ± 7 (range 1–29 years, M : F 78 : 89). Three families were excluded due to non paternity (checked with additional markers) and two families because of ambiguous DQ typing. Table 3 shows the transmission of the DQB1 alleles in the probands in 165 families. Extended TDT analysis showed significant preferential transmission of the HLA-DQ alleles (allele wise TDT $p<0.0001$) (Table 3). DQB1*0201 and DQB1*0302 were the only two alleles to be transmitted in excess to the probands whilst a protective effect was noted only of DQB1*060X after correction for multiple comparisons (where X has not been determined by allele specific primers). The frequency in the probands of HLA-DQB*0201, *0301, *0302, *0303, *0304, *040X, *050X and *060X was 0.35, 0.05, 0.28, 0.03, 0.003, 0.01, 0.15 and 0.12, respectively; 20.7% of probands did not possess either HLA-DQB1*201 or HLA-DQB1*0302.

Glucose tolerance and HLA in parents

HLA-DQB1*0201 showed no correlation with IGT and Type 2 diabetes in parents as shown in Table 4. In comparison, there was a difference between glucose tolerance categories and the presence or absence of DQB1*0302 ($p=0.035$); this may be attributed to a decrease of DQB1*0302 in Type 2 diabetic patients and increase with those with impaired glucose tolerance.

Discussion

The prevalence of Type 2 diabetes (11.2%) and IGT (13.6%) in parents of Type 1 diabetes patients was similar to the prevalence in the general population (Type 2 diabetes 11.6 and 11.2%, respectively) [12]. The mean age at onset of Type 2 diabetes was also similar (43 ± 8.8 years) to that in our previous epidemiological surveys. There was a significantly higher prevalence of Type 2 diabetes in fathers than in mothers (M : W ratio 1.15, $p<0.008$) which was in contradiction to a female excess of the diabetes in the general population (M : W ratio 0.82) [10]. The absence of a higher prevalence of diabetes

Table 3. Transmission for individual DQB1 alleles: χ^2 for allele-wise TDT = 64.7, 7 df, $p<0.0001$; χ^2 for genotype-wise TDT = 72.6, 20 df, $p<0.0001$

	0201	0301	0302	0303	0304	040X	050X	060X
Transmitted	67	10	53	7	1	2	24	16
Not transmitted	20	25	19	18	0	5	39	54
χ^2	25.4	6.4	16.1	4.8			3.6	20.6
p-values	<0.0001	0.01	0.0001	0.03			0.06	<0.0001

040X, 050X, 060X, detailed subtypings were not performed.

Table 4. HLA data in the parents in relation to glucose tolerance

	HLA DQB1 $n=271$			
	*0201		*0302	
Glucose tolerance	Positive $n=119$	Negative $n=152$	Positive $n=88$	Negative $n=183$
NGT	87 (73.1%)	100 (65.8%)	61 (69.3%)	126 (68.9%)
IGT	16 (13.4%)	29 (19.1%)	20 (22.7%)	25 (13.7%)
NIDDM	16 (13.4%)	23 (15.1%)	7 (8.0%)	32 (17.5%)
χ^2	1.93	df=2	6.7	df=2
p	0.38		0.035	

in the mothers, might exclude an '*in utero*' effect in predisposing the offspring for Type 1 diabetes. There was an increased prevalence of IGT in the parents compared to the population data (13.6 and 9.1%) ($\chi^2=6.43$, $p<0.011$). These observations indicated that although the presence of an offspring with Type 1 diabetes did not indicate an increased susceptibility to Type 2 diabetes in the parents, the parents tended to have higher rate of IGT.

Using a family based design the frequency of transmission of the HLA-DQB1 alleles in the Type 1 diabetes probands in this study was similar to our earlier observation in a smaller number of South Indian Type 1 diabetes patients [16,17]. Significant positive association was noted between alleles DQB1*0201 and *0302 and Type 1 diabetes and a negative association with HLADQ1*060X. The HLA associations in the probands also confirmed the clinical diagnosis of Type 1 diabetes. In this ethnic group, as many as 21% of the proband did not possess either DQB1*0201 or *0302. In an earlier study of the probands, we observed that the glutamic acid decarboxylase antibodies ($GAD_{65}Ab$) were positive in 57% and the data was comparable to that in several European Type 1 diabetes subjects [18]. In this study, there appeared to be a correlation between glucose tolerance in the parents with the transmitted HLA-DQB*0302 and glucose tolerance. In the Finnish population, HLA haplotypes associated with Type 1 diabetes susceptibility were found to be predictive of abnormal glucose tolerance in elderly men, with a high sensitivity of 90%, albeit in small numbers [7]. We employed a completely different design based on the transmission disequilibrium test but the study was not powered to examine a specific link between Type 2 diabetes and Type 1 diabetes. Type 2 diabetes in Indians show several differences to those of whites of European ancestry including young age at onset, low rate of obesity, and a high prevalence of insulin resistance [10].

In summary, the presence of Type 1 diabetes in the South Indian offspring does not predict a higher occurrence of Type 2 diabetes in the parents. However, there is an increased occurrence of IGT among the parents. Although some differences were found in the parents between HLA-DQB1*0302 and glucose tolerance, HLA-DQB1 is not a major determinant of glucose tolerance in the parents of Type 1 diabetic offspring.

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