Genetics of Diabetes in Nauru: Effects of Foreign Admixture, HLA Antigens and the Insulin-Gene-Linked Polymorphism

S. W. Serjeantson¹, D. Owerbach², P. Zimmet³, J. Nerup⁴ and K. Thoma⁵

¹Department of Human Biology, John Curtin School of Medical Research, Australian National University, Canberra, Australia,

²Hagedorn Research Laboratory, Gentofte, Denmark, ³WHO Collaborating Centre for the Epidemiology of Diabetes Mellitus, Southern Memorial Hospital, Caulfield, South Victoria, Australia, ⁴Steno Memorial Hospital, Gentofte, Denmark and

⁵Nauru General Hospital, Republic of Nauru, Central Pacific

Summary. Genetic factors play a major role in predisposition to diabetes in the Micronesian population of Nauru. In people aged 60 years and older, 83% of full-blooded Nauruans were diabetic compared with 17% of those with ancestral foreign admixture, as detected by HLA typing. HLA distributions also showed a small increased risk for early onset of diabetes (< 46 years) associated with HLA-Bw22 (Bw56). Variation in the restriction fragment length of DNA near the insulin

gene was found, but was not associated with diabetes. The distribution in fragment lengths, previously reported in Caucasoids, was observed in healthy Polynesians, Melanesians and Micronesians.

Key words: Type 2 diabetes, HLA-Bw22, insulin gene, Nauru, Central Pacific, diabetes genetics.

The genetic component in Type 2 (non-insulin-dependent) diabetes mellitus, in Caucasoids at least, is substantially greater than in Type 1 (insulin-dependent) diabetes [1]. In Type 1 diabetes, specific HLA-antigen associations have been identified in Caucasoids [2, 3], Japanese [3] and Chinese [4], but the major gene or genes predisposing to Type 2 diabetes remain(s) elusive. This is despite reports of minor associations of Type 2 diabetes with HLA-A2 in both the Xhosa in Southern Africa [5] and the Pima of south-western United States [6] and with HLA-Bw61 in Indians from Uttar Pradesh resident in Fiji [7] and South Africa [8]. In Caucasoids, Type 2 diabetes is not associated with HLA antigens but possibly with DNA insertion sequences adjacent to the 5' end of the insulin gene [9, 10, 11]. This observation remains controversial since it has not been confirmed by some workers [12, 13].

We have investigated both HLA and insulin-gene region DNA insertion (deletion) polymorphisms in diabetes in the population of Nauru, a Micronesian group with an adult prevalence of Type 2 diabetes greater than 20% [14]. We have also looked at possible moderating effects of foreign genetic admixture on diabetes in people claiming to be pure Nauruan, but with ancestral foreign admixture as detected by HLA-typing. In addition, we provide, for the first time, population gene frequencies of restriction fragment lengths of the insulin gene in Polynesians, Melanesians and Micronesians.

Subjects and Methods

Ethnographic Background

Nauru is an isolated island in the Central Pacific. Ethnographically, the people are Micronesian with prehistoric genetic affinities with Mongoloid and Melanesian groups [15]. The small population of 4,000 Nauruans, including 2,000 adults, has a limited gene pool since it has contracted markedly even within recorded history. The population was 1476 in 1900 [16] but suffered severe losses due to introduction of infectious diseases, high infant mortality in droughts of 1924–1926 and adult mortality in the Second World War [17].

Thus, the Nauru population has been limited by founder effects, has passed through several genetic bottlenecks and has been discouraged, certainly in recent years, from miscegenation by laws that discourage marriage with non-Nauruans. However, the gene pool is not entirely free of foreign genetic admixture and some of the surnames and HLA haplotypes of early castaways [18] are evident in the modern population of Nauru.

Study Populations

The Nauruans were studied with informed consent during a 3-week period in January, 1982, when adults aged 20 years and older presented at a medical survey that included an oral glucose tolerance test following a 75 g glucose load. The procedures were as described previously for the 1975 medical survey of Nauru [19], except that complete ascertainment of adults was sought and the subgroup selected for genetic studies was drawn from each of the 14 electoral districts.

The prevalence of diabetes is so high in Nauru that suitable controls, who do not have the presumed diabetogenic phenotype, are difficult to identify and any genetic differences in diabetes susceptibility may be manifested in terms of differential ages of onset. Thus, for

Table 1. HLA-A and HLA-B antigen distributions in Type 2 diabetic and non-diabetic subjects from Nauru

HLA antigen	Type 2 diabetic patients: age (years)						Non-diabetic subjects: age (years)					
	36 ± 2 (20–45) (n = 35)		54 ± 1 (46–60) (n = 50)		Total (n = 85)		35 ± 1 (30–45) (n = 31)		54 ± 1 (> 45) (n = 25)		Total (n = 56)	
	No. positive	% positive	No. positive	% positive	No. positive	% positive	No. positive	% positive	No. positive	% positive	No. positive	% positive
A2	5	14.3	4	8.0	9	10.6	1	3.2	5	20.0	6	10.7
Aw24	28	80.0	45	90.0	73	85.9	26	83.9	19	76.0	45	80.4
A26	7	20.0	12	24.0	19	22.4	5	16.1	5	20.0	10	17.9
Aw34	0	0.0	1	2.0	1	1.2	3	9.7	2	8.0	5	8.9
A11	9	25.7	15	30.0	24	28.2	9	29.0	5	20.0	14	25.0
Others	1	2.9	3	6.0	4	4.7	2	6.5	3	12.0	5	8.9
Bw62	7	20.0	19	38.0	26	30.6	15	48.4	9	36.0	24	42.9
Bw39	2	5.7	5	10.0	7	8.2	2	6.5	2	8.0	4	7.1
Bw56	10	28.6	7	14.0	17	20.0	4	12.9	2	8.0	6	10.7
B27	1	2.9	1	2.0	2	2.4	1	3.2	0	0.0	1	1.8
Bw35	13	37.1	23	46.0	36	42.4	16	51.6	6	24.0	22	39.3
B40	19	54.3	31	62.0	50	58.8	17	54.8	18	72.0	35	62.5
Others	2	5.7	3	6.0	5	5.9	2	6.5	2	8.0	4	7.1

Age expressed as mean ± SEM (range)

comparison of HLA antigen and insulin-gene insertion polymorphisms in diabetic and non-diabetic subjects, data have been tabulated for the 30–45 year and 46–60 year cohorts with diabetic patients older than 60 years and non-diabetic subjects younger than 30 years excluded from the comparisons. Further, the control series has been confined to non-diabetic subjects with 2-h post-load plasma glucose concentrations < 8.0 mmol/l. Patients were diagnosed or confirmed as diabetic according to criteria suggested by the WHO Expert Committee on Diabetes Mellitus [20], i.e. fasting plasma glucose ≥ 8.0 mmol/l or with 2-h plasma glucose levels equal to or > 11.0 mmol/l. Thirty-two percent of the sample HLA-typed had diabetic retinopathy. Diabetic patients with and without retinopathy did not differ in HLA distributions or in restriction length phenotypes and have been combined for comparison with non-diabetic subjects.

For analysis of restriction-length fragment polymorphisms of the insulin gene in other Pacific populations, Polynesians from the Wallis Islands, located east of Fiji, and Melanesians from New Caledonia were studied. These subjects were not diabetic according to oral glucose tolerance tests.

Laboratory analysis

HLA-typing was performed in Nauru at 22 °C and according to standard microcytotoxic techniques described elsewhere [21], using 176 antisera to define HLA-A, -B and -C antigens on freshly-drawn lymphocytes. In addition, lymphocytes were frozen within 5 h of collection in liquid nitrogen for subsequent DNA extraction.

DNA was isolated from lymphocytes according to standard procedures [22] and digested with the restriction enzyme Bgl I. DNA fragments were then separated by size on a 1% agarose gel, transferred to commercial filters (Gene Screen, New England Nuclear, Boston, USA) and hybridized with a ³²P-labelled insulin DNA probe [10]. Filters, after washing, were exposed to X-Omat RP film at –70 °C for 8 days. Small Bgl I restriction fragments (3.3 Kb) have been designated L (lower electrophoretic type) and large fragments (> 4.3 Kb) designated U (upper electrophoretic type) following the nomenclature of Owerbach and Nerup [10].

Results

HLA-A and HLA-B antigen frequencies for diabetic and non-diabetic subjects in two age groups are given in

Table 1. The antigen profile is comparatively restricted in full-blooded Nauruans, with only 6 HLA-A and 6 HLA-B locus specificities. Antigens tabulated as 'other' include sporadic instances of A1, A3, A25, A29, Aw31, B7, Bw44, B18 and Bw50. These antigens are unknown in other Pacific populations [23], with the exception of rare instances of B18, and can be assumed foreign to Nauru since they often occurred in known Caucasoid linkage groups such as A25, B18; A29, Bw44 and A3, B7.

The most appropriate comparison for examining HLA-associated age-of-onset effects is between young diabetic patients (< 46 years) and older non-diabetic subjects (≥ 46 years). Comparison of these two groups shows no differences in HLA-A locus antigen distributions, but at the HLA-B locus there is an increase in frequency of Bw22 (Bw56) in diabetic patients ($\chi^2 = 3.86$, $p = 0.05$). This difference is not significant when comparisons are made within age groups nor when age groups are combined. The increase in Bw22 in young diabetic patients is accompanied by a decreased frequency of Bw62 which is statistically significant when compared with young non-diabetic subjects ($\chi^2 = 5.96$, $0.01 < p < 0.05$) and with the total control series ($\chi^2 = 5.01$, $0.01 < p < 0.05$).

Table 2 gives the distributions of individuals with foreign HLA types according to glucose tolerance test results. Individuals with foreign antigens at both the HLA-A and HLA-B loci are included only once in Table 2. The highest proportion of detectable admixture occurred in those with impaired glucose tolerance (2-h post-load plasma glucose: 8.0–10.9 mmol/l), significantly higher than in diabetic patients ($\chi^2 = 7.80$, $p < 0.01$) but not different from those with normal glucose tolerance. The divergent risks for diabetes in those with and without foreign admixture are most marked in

Table 2. The distributions of individuals with foreign HLA types according to diabetic, impaired and normal glucose tolerance test results

Age (years)	Diabetic patients		Impaired glucose tolerance		Normal glucose tolerance		Relative risk ^a	
	Number tested	Foreign HLA (%)	Number tested	Foreign HLA (%)	Number tested	Foreign HLA (%)	RR	p
20–45	34	5.9	17	11.8	74	12.2	1.86	NS
46–59	46	8.7	9	22.2	22	9.1	1.22	NS
60+	20	5.0	6	66.7	3	33.3	4.96	0.003
Total	100	7.0	32	25.0	99	12.1	1.76	0.054

^a Risk for diabetes if pure Nauruan ancestry compared with risk if foreign admixture; NS = not significant

Table 3. Distribution of UU, UL and LL genotypes in Type 2 diabetic and non-diabetic subjects from Nauru

Age (years)	Type 2 diabetic patients							Age (years)	Non-diabetic subjects						
	Total	UU		UL		LL			Total	UU		UL		LL	
	No.	No.	%	No.	%	No.	%		No.	No.	%	No.	%	No.	%
								20–29	26	0	0.0	8	30.8	18	69.2
20–45	19	0	0.0	7	36.8	12	63.2	30–45	19	1	5.3	8	42.1	10	52.6
46–60	30	2	6.7	12	40.0	16	53.3	> 45	15	0	0.0	5	33.3	10	66.7
> 60	9	0	0.0	5	55.5	4	44.4								
≤ 60	49	2	4.1	19	38.8	28	57.1	≥ 30	34	1	2.9	13	38.2	20	58.8
All ages	58	2	3.4	24	41.4	32	55.2	All ages	60	1	1.7	21	35.0	38	63.3

Table 4. Distribution of U and L phenotypes and allele frequencies in normal subjects from four ethnic groups

Ethnic group	UU		UL		LL		U allele	
	No.	%	No.	%	No.	%	Frequency	Variance
Micronesian (n=60)	1	1.7	21	35.0	38	63.3	0.192	0.0013
Polynesian (n=46)	6	13.0	18	39.1	22	47.8	0.326	0.0024
Melanesian (n=36)	2	5.6	4	11.1	30	83.3	0.111	0.0014
Caucasoid [24] (n=52)	2	3.8	24	46.2	26	50.0	0.269	0.0019

those aged 60 years and older. In this age group, 95% of diabetic patients had full-blooded Nauruan ancestry on the basis of HLA typing, compared with 44% of non-diabetic subjects. The only diabetic patient aged over 60 years and with foreign admixture was a 75-year-old woman with 2-h plasma glucose concentration of 13.2 mmol/l.

The distributions of UU, UL and LL genotypes in unrelated diabetic patients are given in Table 3 and are compared with non-diabetic subjects with normal glucose tolerance. There are no differences in genotype distributions in diabetic and non-diabetic subjects in any age group. The diabetic sample aged 60 years or younger includes three people with detectable foreign admixture and the control series (≥ 30 years) five subjects with a foreign HLA haplotype.

Table 4 provides the frequencies of the U allele in normal subjects in three Pacific populations for com-

Fig. 1. Autoradiogram of insulin-gene-containing DNA sequences in DNA digested with Bgl I from a Polynesian family. (1) Wife, (2) husband, (3) child 1, (4) child 2, (5) child 3, (6) child 4, (7) child 5

parison with the published frequency [24] for normal Caucasoids. Only the Melanesian frequency is different from that in Caucasoids ($\chi^2 = 6.63$, $p = 0.01$).

The sizes of the U and L insertions in Pacific populations are also comparable with those observed in Caucasoids [10]. In Caucasoids, the U allele ranges in size from 4.3–5.0 kilobases (Kb) compared with 4.4–5.6 Kb in Melanesians. The L alleles can range in size in Caucasoids from 2.7–3.2 Kb and varied between 2.7–2.9 Kb in all Pacific groups, with the exception of two examples in Nauru of L insertions of 3.3 Kb.

Figure 1 shows an autoradiogram, after hybridization with the ³²P-labelled insulin probe, of U and L insertions in a Polynesian family. Both parents are heterozygous UL, but have different sizes of the L insertion as is apparent on the autoradiogram and confirmed in child number 5, who is heterozygous for two L subtypes. Child number 1 is heterozygous UL and children

numbers 2, 3 and 4 are homozygous UU, clearly showing the Mendelian inheritance of U and L insertions in this Polynesian family.

Discussion

We have provided the first evidence for a genetic component in Type 2 diabetes in the Pacific by finding, in the population of Nauru, that ancestral foreign admixture has a protective effect against diabetes. This is most marked in the older generations, possibly reflecting a lesser diluting of imported genes and concomitantly, more accurate detection of foreign admixture by HLA typing. Also, in the older generations, a higher proportion of persons with the diabetogenic phenotype are likely to be detected by glucose tolerance tests.

In the generation aged 60 years and older, 83% of full-blooded Nauruans in the genetic sample had diabetes, compared with only 17% of part-Nauruans. That this difference is due to genetic influences seems indisputable, since there is no evidence today to suggest differential diets or life-styles of part and full-blooded Nauruans. In addition, body mass index, a measure of obesity defined as weight (kg) divided by the square of height (metres) did not differ between part and full-blooded men or women.

The highest proportion of foreign admixture occurred in those with impaired glucose tolerance, suggesting a moderating codominant effect of foreign genes at one or more loci. However, the great majority of Nauruans have no European ancestry and most of these have diabetes by age 60 years. This suggests that the genes predisposing to diabetes, whether through selection or genetic drift, have attained extraordinarily high frequencies. Given the extreme geographical isolation of Nauru and severe constrictions in population size, the genetic profile of Nauruans may well reflect the impact of genetic drift. Indeed, there is, for instance, a high frequency (7%) of $C6^{\text{Nauru}}$ [25], an iso-electric variant of the complement component C6 that is rare or absent in other populations. Also, a variant of erythrocyte glyoxalase attains polymorphic frequencies in Nauru [26] although it is completely absent from ten other Asian and Pacific populations studied. The effects of selection, however, in contributing to uniquely high frequencies of a diabetogenic phenotype, cannot be discounted since the impact of drought and famine on infant mortality is well-documented for the island [17], with much of the mortality attributed to vitamin B1 deprivation [27].

The genes predisposing to diabetes are so ubiquitous in full-blooded Nauruans that our studies focussed on possible genetic differences in age of onset of the disease, by studying two genomic segments claimed to be associated with Type 2 diabetes in other ethnic groups.

In the major histocompatibility complex there was a small but significant increase in HLA-Bw22 in younger diabetic patients. The significance of this association has not been corrected for the number of antigens tested, due to a priori assumptions based on our previously observed significant increase in HLA-Bw22 in Polynesian diabetic patients [28]. This antigen may well be associated with a real but small increase in risk for Type 2 diabetes in the Pacific.

The second genetic system examined, variation in the Bgl I restriction fragment lengths containing the insulin gene, showed no association with age-of-onset of diabetes in Nauru. There were, however, two distinct size classes of alleles: Bgl I restriction fragments of 2.7–3.3 Kb (L-alleles) and 4.4–5.0 Kb (U-alleles). No Bgl I sequences were found between 3.3 and 4.4 Kb. Indeed, the bimodal distribution of restriction fragments has been maintained in an outbred Caucasoid population [24], Melanesians and Polynesians as well as in Micronesians. A regulatory role for U alleles on glucose concentration has been suggested in the Caucasoid population [29]. In Micronesians, they may also play a regulatory role, though they are currently undetected because of the effects of other more dominant genes.

Our data suggest a genetic aetiology in the Type 2 diabetes of Nauru. The data support earlier claims of minor MHC influences in Type 2 diabetes in some ethnic groups [6, 7], especially in age-of-onset effects and suggest, by the clear absence of a diabetes association, with the U and L restriction fragment types containing the insulin gene that the genes leading to diabetes in Nauru may differ from those claimed to lead to Type 2 diabetes in some Caucasoids [10, 11].

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Dr. S. W. Serjeantson
Department of Human Biology
John Curtin School of Medical Research
Australian National University
P.O. Box 334
Canberra, ACT
Australia