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Analysis of HLA DQA1 and DQB1 alleles and glycated hemoglobin in healthy Mexican mestizo individuals from families with type 1 diabetes mellitus.

Original Article

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SUMMARY.

Introduction. Variations in hemoglobin (HbA_1) levels in healthy individuals are attributable to complex individual biological differences. Specific HLA DQ genes are known to predispose subjects to the development of type 1 diabetes mellitus (DM1).

Material and methods. HLA DQA1 and DQB1 genes and HbA_1 heritability were investigated in healthy individuals from DM1 and non-DM1 Mexican families. HbA_1 levels were determined by ion-exchange chromatography and HLA class II alleles were typed by PCR, in 77 healthy persons from 18 families with at least one member suffering from DM1 (DM1R group) and in 96 healthy individuals from 18 families with no known history of DM, who constituted the control group.

Results. There were no significant inter-group differences in HbA_1 levels. Mean HbA_1 levels were correlated between parents and offspring, and the heritability estimate was 53% based on a regression model. DQA1 and DQB1 typing revealed inter-group differences, with an increase in the susceptibility allele, DQB1 *0201, in the DM1R group relative to the control group (17.24% vs 3.23%, respectively; $p < 0.05$), as expected. HbA_1 levels did not correlate with HLA DQA1 or DQB1 polymorphisms.

Discussion. The present data suggest that 53% of HbA_1 variability is explicable by genetic factors, which do not depend on DQA1 or DQB1 polymorphisms. ***(Rev Biomed 2003; 14:125-130)***

Key words: Glycated Hb, type 1 diabetes mellitus, protection alleles, DQA1 genotype, DQB1 genotype.

RESUMEN.

Análisis de los alelos HLA DQA1 y DQB1 y hemoglobina glucosilada en personas mestizas de familias con diabetes mellitus tipo 1.

Introducción. Las variaciones de HbA_1, en individuos sanos, son atribuidas a diferencias biológicas

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complejas. Se conoce que los genes HLA DQ participan en la predisposición para desarrollar diabetes mellitus tipo 1 (DM1).

Material y métodos. Los genes HLA DQA1 y DQB1 y la heredabilidad de los niveles de HbA_1, se investigaron en individuos sanos de familias mexicanas con DM1 y sin antecedentes de diabetes. La HbA_1 se determinó por cromatografía de intercambio iónico y los alelos HLA clase II se tipificaron por técnicas de PCR en 77 personas sanas de 18 familias con al menos un caso índice con DM1 (DM1R) y 96 individuos de 18 familias sin antecedentes familiares de DM como grupo control.

Resultados. No se observaron diferencias en los niveles de HbA_1, entre ambos grupos. Con base en el análisis de coeficiente de regresión, se correlacionaron HbA_1 entre padres e hijos y se estimó una heredabilidad de 53%. El análisis de HLA entre los grupos, mostró, como era esperado, incremento de alelos de susceptibilidad en el grupo DM1R (DQB1 *0201 17.24 % VS 3.23 %, $p < 0.05$). No se observó relación entre los niveles de HbA_1 y los polimorfismos encontrados.

Discusión. Los datos del presente trabajo sugieren que la variabilidad de la HbA_1 es independiente de los polimorfismos DQA1 y DQB1, pero que en un 53% se explica por un componente genético.

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Palabras clave: Hemoglobina glucosilada, diabetes mellitus tipo 1, alelos de protección, genotipo DQA1, genotipo DQB1.

INTRODUCTION.

The level of glycated hemoglobin ($HbA_1 = HbA_{1a} + HbA_{1b} + HbA_{1c}$) has been a useful index in the assessment of glycemic control in DM (1,2), and shows biological variability (3,4). Several factors, including some unrelated to glucose metabolism**,** have been suggested to contribute to HbA_1 variability (4). However, diabetes-related conditions are the only well-recognized factors that influence HbA_1 levels (5). Conversely, HbA_1 heritability has been estimated by monozygotic and dizygotic twin data analysis to be approximately 62% (6). Insulin-dependent diabetes mellitus or type 1 diabetes mellitus (DM1) is inherited as a complex polygenic trait (7). Together with other genes (7–9), the HLA class II genes are major contributing factors in the pathogenesis of DM1 (8,9). The genes encoding HLA DQ molecules have been strongly correlated with DM1 (10,11), so they are recognized as principal determinant factors of this disease (10). Susceptibility to DM1 is associated with Ala, Val or Ser at position 57 of the DQb chain and protection from DM1 by the presence of aspartic acid in this position, together with the presence (susceptibility) or absence (protection) of arginine at position 52 of the DQa chain (10–12).

In this study, HbA_1 heritability and the contribution made by the HLA class II DQA1 and DQB1 alleles to HbA_1 levels were investigated in healthy individuals from 18 DM1-affected and 18 non-DM1-affected Mexican Mestizo families.

MATERIALS AND METHODS.

Two groups of healthy adult individuals were studied. The first group consisted of 77 relatives from 18 DM1 families (29 parents and 48 siblings) associated with la Unidad de Endocrinología del Hospital de Especialidades, Centro Médico Nacional de Occidente, IMSS (group DM1R). The second group comprised 96 healthy volunteers (31 parents and 65 offspring) from 18 families with no known history of DM1 or DM2, living in the Guadalajara metropolitan area (group C). No parental consanguinity was reported in any family.

HbA_1 levels were determined by ion-exchange chromatography (Sigma Diagnostics, St. Louis, MO, USA). Genomic DNA was obtained from peripheral blood by standard techniques (13,14).

HLA typing. DNA encoding the variable regions of the second exons of DQA1 and DQB1 was amplified using sequence-specific oligonucleotide probes (SSOP) and samples from five DM1R and five C group families, according to the protocol described at the 11^{th} and 12^{th} International Histocompatibility Workshops (15,16). Nineteen probes were used for

Table 1
Blood HbA_1 concentrations in random individuals from DM1R and C groups.

Individuals	DM1R Mean %, SD (n)	C Mean %, SD (n)
Parents	6.34, 1.2 (29)	6.58, 0.95 (31)
Offspring	6.12, 0.98 (48)	6.27, 0.92 (65)
Total	6.20, 1.07 (77)	6.37, 0.94 (96)

the DQA1 alleles and 22 for the DQB1 alleles. The probes were labeled with 11-digoxigenin-UTP, using the same protocols (15,16). Hybridizations were visualized by chemiluminiscence, with the anti-digoxigenin antibody, CSPD (Boehringer Mannheim), and exposed to x-ray films (Kodak X-OMAT KXK-1) according to Zetterquist and Olerup (1992) (17).

Advances in the molecular biology techniques available in our laboratory allowed us to use sequence-specific primer (SSP) technology to analyze the remaining families (13 from group DM1R and 13 from group C). We also re-analyzed some samples previously processed with the SSOP technique to confirm those results.

Sequence-Specific Primers. Twenty-two primers were combined into 14 primer mixes and used to amplify the DQB1 alleles; 13 primers (10 mixes) were used to amplify the DQA1 alleles. Two additional primer mixes were used to discriminate between the DQA1*0101 and *0104 alleles. Each primer-pair combination was tested against positive and negative control DNA. A primer pair that amplifies a 796-bp fragment from the third intron of DRB1 was included as an internal positive control (17,18).

To analyze the distribution of susceptibility (S) and protection (P) alleles, both parents from each family (independent chromosomes) were considered. Twenty-nine subjects (2n = 58) were studied in the DM1R group and 31 in the C group (2n = 62). According to the presence or absence of Arg52 in DQA1 and Asp57 in DQB1, the S alleles were DQA1*0301, *0401, and *0501, and DQB1*0201, *0302, and *0501; the P alleles were DQA1*0101, *0102, *0103, *0104, and *0201, and DQB1*0301, *0402, *0602, and *0603. The alleles were pooled according to this classification, and an inter-group comparison was made. Intra-group percentile conversion of HbA1 was used to generate a contingency table to test the correlation between high or low HbA1 levels and S or P allele distributions.

Statistical Analysis. Means and standard deviations (SD) of the quantitative variables are described for every group. Inter-group and parent–sibling comparisons were made of HbA_1 values, using ANOVA and Student's *t*-test respectively . Genotype and allele frequency distributions for the susceptibility and protection alleles were analyzed by contingency tables (Hardy – Weinberg equilibrium). Heritability (h^2) was estimated from the regression of offspring on parental HbA_1 values (19). SPSS 10.0 software package (SPSS Inc., Chicago, IL, USA) and simulation computer programs based on the c^2 and Fisher's exact tests were used for analysis.

RESULTS.

The means and SD for HbA_1 levels are presented by group in Table 1, and the regression coefficients of offspring on parents are given for HbA_1 levels in Table

Table 2
HbA_1 offspring–parent regression coefficients, p values (number of pairs).

	P1	SM	S1	S2	S3
PM		0.53, 0.001 (36)	0.33, 0.050 (36)	0.28, 0.098 (35)	0.41, 0.103 (17)
P1		0.78, 0.000 (28)	0.78, 0.000 (28)	0.49, 0.009 (27)	0.70, 0.005 (14)
P2	0.25, 0.24 (24)	0.46, 0.008 (32)	0.35, 0.049 (32)	0.42, 0.018 (31)	0.45, 0.100 (14)

PM = parental mean; **P1** = Father; **P2** = Mother; **SM** = siblings mean; **S1** = first son, sibling 1; **S2** = second son, sibling 2; **S3** = third son, sibling 3.

Table 3
HLA-DQA1 and HLA-DQB1 genotype distributions by group (counts).

DQA1 GENOTYPE	DM1R (n = 29)	C (n = 31)	DQB1 GENOTYPE	DM1R (n = 29)	C (n = 31)
*0101/*0102	0	1	*0201/*0301	0	1
*0101/*0301	1	2	*0201/*0302	2	1
*0101/*0302	0	1	*0201/*0402	2	0
*0101/*0501	1	0	*0201/*0501#	5	0
*0101/*0103	0	1	*0201/*0602	1	0
*0102/*0301	2	3	*0301/*0301	1	3
*0102/*0401	1	0	*0301/*0302	6	3
*0102/*0501	2	1	*0301/*0402	0	1
*0104/*0201	0	2	*0301/*0501	0	2
*0104/*0301	1	3	*0301/*0601	0	1
*0104/*0401	1	0	*0301/*0602	1	2
*0104/*0501	3	0	*0302/*0302	0	1
*0201/*0301	0	1	*0302/*0402	0	3
*0301/*0301	2	3	*0302/*0501	3	5
*0301/*0302	1	0	*0302/*0602	7	6
*0301/*0401#	1	6	*0501/*0601	0	1
*0301/*0501	10	6	*0501/*0602	1	1
*0302/*0302	0	0			
*0302/*0401	1	0			
*0401/*0501	1	0			
*0501/*0501	1	1			

# $p < 0.01$

2. No significant differences in HbA_1 levels were observed between groups (*t*-test or ANOVA). Although the parental (father–mother) HbA_1 regression coefficient was 0.25 ($p = 0.24$, 24 pairs), statistically significant parent–offspring regression coefficients were observed (table 2).

HLA DQA1 and DQB1 analyses. Genotype and allele distributions are presented in tables 3 and 4.

Comparison of the observed DQA1 and DQB1 genotype distributions in the C group were similar to those predicted under Hardy–Weinberg equilibrium ($p > 0.3$). Genotype comparisons between groups showed statistically significant differences ($p < 0.01$) only for DQB1*0201/*0501 (S/S) and DQA1 *0301/*0401 (S/P), with the former displaying an increased frequency and the latter a decreased frequency in the DM1R group. The allele frequency distribution differed only for DQB1*0201, which was elevated in the DM1R group ($p < 0.05$). The results of allele recoding in terms of S or P equivalence are presented in table 5. After S and P were pooled, no significant between-group differences were observed.

DISCUSSION.

Blood HbA_1 levels are a reliable predictor of DM in healthy people (21). Apart from DM-related conditions, it is important to consider non-diabetic factors in determining the biological variability of HbA1 in healthy individuals (3, 4) and to specifically investigate the genetic factors involved in this variability (6, 20).

In the present study, HbA_1 levels were normally distributed and no significant inter-group differences were observed. However, the absence of father–mother correlations and the significant parent–offspring correlations indicate a genetic component in HbA_1 variability. Heritability was calculated to be 53% on the basis of offspring on parental mean HbA_1 levels (table 2). HbA_1 heritability estimated in a twin study

Table 4
DQA1 and DQB1 allele frequencies by group.

Allele	DM1R (2n=58) count	%	C (2n=62) count	%
DQA1				
*0101 (P)	4	6.80	4	6.45
*0102 (P)	8	13.78	6	9.68
*0104 (P)	5	8.61	5	8.06
*0201 (P)	0	0.00	3	4.84
*0301 (S)	17	29.30	27	43.55
*0302 (S)	2	3.43	2	3.22
*0401 (S)	5	8.78	6	9.68
*0501 (S)	17	29.30	9	14.52
*0601 (P)	0	0.00	0	0.00
Total	**58**	**100.00**	**62**	**100.00**
DQB1				
*0201 (S)[#]	10	17.24	2	3.23
*0301 (P)	9	15.52	15	24.19
*0302 (S)	18	31.03	20	32.26
*0402 (P)	2	3.45	4	6.45
*0501 (S)	9	15.52	9	14.52
*0601 (P)	0	0.00	2	3.22
*0602 (P)	10	17.24	10	16.13
Total	**58**	**100.00**	**62**	**100.00**

[#] $p < 0.05$, inter-group comparison.

was 62%, suggesting that genetic factors influence the glycation of proteins by glucose-independent mechanisms (6). Our data reinforce the observations made in monozygotic and dizygotic twins, confirming that hereditary factors are involved in determining blood levels of HbA$_1$.

With respect to HLA polymorphisms, the comparison of genotype distributions showed an increased frequency of DQB1*0201/*0501 in the DM1R group relative to the control group (5 *vs* 0, respectively; $p < 0.01$). This was expected, since the genotype is composed of two S alleles. However, DQA1 *0301/*0401, an S/P genotype, occurred at a reduced frequency in the DM1R group relative to the control group (1 *vs* 6, respectively; $p < 0.01$). On the other hand, when allele frequencies were compared, only DQB1*0201, a DM1 susceptibility allele, was found at elevated levels in the DM1R group relative to the control group (17.24% *vs* 3.23%,

Table 5
S and P alleles in both groups.

	n[#] (%) S*	n (%) +P*	n (%) Total
Total DMR1	**73 (63)**	**43 (37)**	**116(100)**
Total C	**69 (56)**	**55 (44)**	**124(100)**

* includes DQA1+DQB1 alleles.
[#] chromosomes

respectively; $p < 0.05$). Therefore, in the study population, the observed susceptibility alleles were similar in families with DM1 from northwestern Mexico to those described in other populations (11,12, 22, 23), with substantial increases in the *0501 allele of DQA1, although this was not statistically significant, and in the *0201 allele of DQB1 (Table 4). The DQB1 *0201 allele has been previously identified as significantly increased in Mexican Mestizo individuals with DM1 (22, 23).

Because no differences were found in an inter-group comparison of mean HbA$_1$ levels, and no inter-group differences were observed between the DM1R and C groups in terms of DQA1 and DQB1 alleles typing, we conclude that these HLA class II genes do not influence HbA$_1$ levels and are therefore not involved in the biological variability of HbA$_1$.

In summary, the present data indicate that HbA$_1$ levels were similar in healthy individuals from the DM1R and C groups, that these levels were not associated with DQA1 and DQB1 polymorphisms, and that 53% of HbA$_1$ variability can be explained by genetic factors.

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