Increase in Capillary Basement Membrane Width in Parents of Children with Type I Diabetes Mellitus

Association with HLA-DR4

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SUMMARY

Hereditary factors related to HLA antigens are known to play a role in determining risk for the development of type I diabetes. In the present investigation, asymptomatic first degree relatives of diabetic patients were investigated to determine whether any abnormalities could be associated with the HLA haplotypes. Muscle biopsies were performed to obtain measurements of the width of the capillary basement membranes in 16 type I diabetic children, 20 of their unaffected siblings, and 38 parents. Only two diabetic children had capillary basement membranes greater than 2000 Å. The mean width of the capillary basement membranes was not different in affected compared with unaffected siblings. In contrast, the capillary basement membranes in the parents were considerably larger, with 14 of the 38 parents (37%) having measurements greater than 2000 Å. Moreover, the width of the capillary basement membranes in the parents correlated with the presence of the antigen HLA-DR4. The mean capillary basement membrane width in DR4 positive parents was 2026 ± 350 Å; that of DR4 negative parents was 1642 ± 333 Å. The difference was highly significant ($P < 0.001$). There was no correlation of capillary basement membrane width with HLA-DR3. The results suggest that a risk factor for type I diabetes associated with HLA-DR4 was associated in parents of type I diabetic patients with an asymptomatic increase in capillary basement membrane width in the absence of any clinical evidence of diabetes. The possible role of these abnormalities in the pathogenesis of type I diabetes and of the vascular complications of the disease require further study.

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Type I diabetes is known to be associated with an abnormal distribution of certain HLA antigens,[1–11] primarily with the antigens DR3 and DR4.[3–11] The previously noted increases of B8 and B15 appear to be due to linkage disequilibrium within the HLA system. The common haplotypes are B8,DR3 and B15,DR4. An excess of DR3,DR4 heterozygotes exists, suggesting that perhaps the two genes have an additive effect in determining risk for type I diabetes.[9]

In a number of studies,[12–16] patients with diabetes have been found by electron microscopy to have an increased quadriceps capillary basement membrane width. Siperstein and co-workers[12] reported that increases in skeletal muscle capillary basement membrane width were present in approximately one-half of adult asymptomatic offspring of two overtly diabetic parents. Such thickening of capillary basement membranes in unaffected relatives of patients with diabetes was thought to be due to genetic factors.[12]

In the present investigation, we performed HLA typing and obtained muscle biopsies to determine the width of the capillary basement membranes in 22 families of Caucasian children with type I diabetes. Abnormally wide capillary basement membranes were found in some of the parents who did not themselves have clinical diabetes. This thickening of the capillary basement membranes was strongly correlated with the presence of HLA-DR4.

MATERIALS AND METHODS

We studied 22 families in which at least one child had type I diabetes mellitus. Included were 28 patients, 29 unaffected siblings, and 38 unaffected parents. Unaffected siblings and parents did not have any signs or symptoms of clinical diabetes and had fasting blood sugar concentrations from 60 to 100 mg/dl. All but one of these individuals had normal responses to a 100-g oral glucose load as defined by the National Diabetes Data Group.[17] One mother had a fasting blood sugar of 69, peak sugar of 229, and 2-h sugar of 216 mg/dl, but suppressed glucagon to 51 pg/ml and increased

insulin to 130 μU/ml following the glucose load. Thus, she had an abnormal glucose tolerance test[17] but a normal hormonal response to a glucose load.[18] Three additional parents, known to have type I diabetes, were excluded from the analysis. Ten normal nondiabetic adult subjects served as controls for measurement of quadriceps muscle capillary basement membranes. These normal subjects had no family history of diabetes, and had fasting blood glucose concentrations from 60 to 100 mg/dl and normal responses to a standard glucose load. All of the subjects were Caucasoids residing in the Dallas area.

Quadriceps muscle biopsies were performed under local anesthesia with a Franklin-Silverman biopsy needle. The pieces of tissue were immediately fixed in osmic acid and processed according to methods described by Siperstein and co-workers.[12,13] The basement membrane was measured in 20 equidistant places around the periphery of each vessel and the average of 30 capillaries was calculated for each subject. Sixteen patients with type I diabetes, 20 unaffected siblings, and 38 unaffected parents each had a biopsy performed.

HLA-A,B and C typing was performed using the NIH microcytotoxicity procedure.[19] Sera used for these determinations permitted identification of all WHO-IUIS specificities. The sera originated from our own serum procurement program, from exchanges with other investigators, and from the NIH serum bank. Typing for the HLA-DR specificities was performed by cytotoxicity with isolated bone marrow derived (B) lymphocytes obtained from peripheral blood by nylon-wool filtration.[20,21] The sera used for defining HLA-DR specificities were of local origin and obtained through exchange with other investigators. Twenty-eight patients with type I diabetes, 29 unaffected siblings, and 38 parents had HLA typing performed. Eighty-five unselected Caucasian normal subjects who have had HLA typing performed for other purposes during the past year form the basis of the control group of HLA frequencies.

Calculations to determine association between HLA antigens and quadriceps muscle capillary basement membrane width were performed using the Chi square test with Yates' correction and Fisher's exact test. The differences between means were evaluated using Student's *t* test. Covariance analysis was determined by the method of Zar.[22]

RESULTS

Capillary basement membrane width in normal adults. Quadriceps biopsies were performed on 10 normal subjects between the ages of 28 and 50 yr. The capillary basement membrane width was 1628 ± 218 Å (mean ± SD) (Table 1). All 10 persons had normal glucose tolerance tests.

TABLE 1
Capillary basement membrane width in normal adult subjects

Subjects	Age (yr)	Sex	CBMW* (Å) mean ± SD
P.T.	30	M	1761 ± 188
T.H.	30	M	1924 ± 130
H.D.	30	M	1618 ± 105
L.L.	36	F	1447 ± 148
P.A.	28	F	1945 ± 189
K.T.	33	F	1371 ± 134
S.M.	32	F	1264 ± 161
J.J.	43	F	1619 ± 134
T.L.	38	M	1536 ± 69
R.T.	50	M	1801 ± 185
Mean ± SD	35 ± 7		1628 ± 218

* CBMW, capillary basement membrane width.

Capillary basement membrane width and HLA typing in children with type I diabetes. Two of the 16 offspring with diabetes who had muscle biopsies were found to have capillary basement membrane width greater than 2000 Å. Both patients were 19 yr old or older at the time of study. Their HLA types were A1,A11,B7,B8,C-,DR3,DR4 and A2,A29, B15,Bw44,Cw3,DR2,DR7. The other 14 children were all 18 yr old or younger at the time of the study. Children with type I diabetes showed increased frequency of DR4 and a decreased frequency of DR2 (Table 2).

Quadriceps basement membrane width and frequency of HLA antigens in siblings of children with type I diabetes. None of the 20 unaffected siblings who had quadriceps biopsies had a capillary basement membrane width greater than 1800 Å. The DR frequencies in the 29 unaffected siblings studied showed no difference in any of the eight types studied in comparison with the control Caucasian population (Table 2).

Frequency of HLA-DR antigens and capillary basement membrane width in parents of children with type I diabetes. Data for the 38 parents, including age, sex, results of HLA-A,B,C, and DR typing, and capillary basement width measurements are shown in Tables 3 and 4.

None of the parents included in the study had evidence of diabetes, although one parent had an abnormal glucose tolerance test, as described in METHODS. Three additional parents, not included in the analysis, who did have diabetes of long duration, had thickened capillary basement membranes. Their HLA types were Aw24,B15,Bw33,Cw1,Cw3,

TABLE 2
HLA-DR antigens in children with type I diabetes and first degree relatives

Subjects	Number tested	DR1	DR2	DR3	DR4	DR5	DRw6	DR7	DR8
Normal unrelated	85	19	31	24	29	7	14	19	7
Children with diabetes	28	10	7*	43	64†	0	21	7	0
Unaffected siblings	29	14	28	34	48	3	24	7	0
Parents	38	5	21	39	53*	11	18	13	0
Parents with CBMW < 2000 Å	24	8	21	38	33	14	14	7	0
Parents with CBMW > 2000 Å	14	0	21	43	86‡	14	14	7	0

* $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$. CBMW, capillary basement membrane width.

TABLE 3
HLA-DR4 positive parents

Subject	Age (yr)	Sex	A	B	C	DR	CBMW* (Å) Mean ± SD
M.R.	33	F	3,11	15,22	w3,—	4,w6	1911 ± 83
K.P.	45	M	1,3	17,40	w6,—	4,7	1657 ± 112
C.P.	45	F	1,2	8,15	w3,—	3,4	2064 ± 245
B.W.	46	M	2,26	12,w31	—,—	4,5	29.6 ± 201
J.W.	45	F	26,—	7,27	w2,—	4,w6	2170 ± 210
J.H.	35	M	2,11	15,w35	w3,w5	2,4	1744 ± 74
S.L.	44	F	1,11	8,—	w6,—	3,4	2116 ± 221
J.P.	50	M	3,w24	7,w51	—,—	2,4	1865 ± 142
M.P.	47	F	2,11	15,51	w3,—	4,w6	2149 ± 72
G.P.	37	M	2,—	12,15	w3,—	4,—	2019 ± 119
J.M.	58	F	2,w24	7,w35	w4,—	2,4	2271 ± 213
W.C.	50	M	3,w24	7,14	—,—	2,4	2348 ± 228
N.C.	44	F	1,3	12,—	w5,w6	3,4	1255 ± 132
S.F.	45	F	1,2	w35,w38	w4,—	4,—	2032 ± 100
W.B.	39	M	1,w31	8,40	w3,—	3,4	2183 ± 200
J.B.	38	F	w24,29	12,w39	—,—	2,4	2026 ± 123
P.H.	41	F	1,2	8,w39	—,—	3,4	1806 ± 170
M.G.	35	M	2,w31	5,40	w3,—	3,4	2492 ± 134
H.R.	29	M	3,w23	12,15	w3,w4	4,7	1609 ± 175
M.M.	37	F	2,—	5,15	w1,—	4,5	1890 ± 176
Mean ± SD	42.2 ± 6.8						2026 ± 350

* CBMW, capillary basement membrane width.

DR3,DR4; A2,A29,B12,B15,Cw3,DR1,DR4; and A2,A11, B12,Bw35,Cw4,DR1,DR4.

There were 24 parents with capillary basement membrane widths of less than 2000 Å. Their HLA-DR frequencies were no different from those of normal unrelated Caucasian controls (Table 2). There were 14 parents with capillary basement membrane widths of greater than 2000 Å. This group has a frequency of DR4 of 86%, significantly different ($P < 0.001$) from the frequency of controls, which was 29% (Table 2). The difference is significant also if corrected for the number of antigens tested ($P_c < 0.008$). The frequency of HLA-DR4 in the total group of parents was 53%, significant only at the level of $P < 0.05$ (Table 2). In the DR4 positive parents the capillary basement membrane width was 2026 ± 350 Å, which was significantly different from that of the DR4 negative parents, 1642 ± 333 Å ($P < 0.001$), using the Student's *t* test (Figure 1). The mean age for the DR4 positive parents was 42.2 ± 6.8 yr, and for the DR4 negative group, 41.6 ± 7.8 yr. This difference was not statistically significant. The sex distribution in both groups was similar. Capillary basement widths greater than 2000 Å were observed in 12 of the 20 DR4 positive parents and only 2 of the 18 DR4 negative parents ($P < 0.005$) (Table 5). There was no statistically significant relationship for DR3 and capillary basement membrane width in the parents.

The difference in capillary basement membrane width be-

TABLE 4
HLA-DR4 negative parents

Subject	Age (yr)	Sex	A	B	C	DR	CBMW* (Å) Mean ± SD
J.A.	35	F	2,—	8,40	—,—	3,—	1361 ± 128
J.W.	41	M	3,w24	7,—	—,—	2,3	1920 ± 104
B.W.	41	F	1,2	8,w39	—,—	3,—	1367 ± 117
J.R.	36	M	2,—	12,15	w3,w5	—,—	1489 ± 96
L.H.	34	F	w24,w30	15,18	w3,—	3,5	2372 ± 266
N.L.	48	M	1,—	15,w51	w2,—	w6,—	1874 ± 179
J.T.	38	M	2,3	15,w39	w3,—	1,—	1366 ± 88
F.T.	34	F	1,w24	8,w39	—,—	3,—	1041 ± 80
M.P.	36	F	2,3	27,w35	w2,w4	7,—	1809 ± 98
K.M.	58	M	11,28	8,12	—,—	3,7	2302 ± 169
V.H.	53	M	2,11	8,12	—,—	3,—	1358 ± 66
M.H.	44	F	2,—	8,w22	w3,—	26,—	1589 ± 95
J.B.	42	M	2,3	7,15	w3,—	2,—	1577 ± 82
K.B.	35	F	1,2	8,40	w3,—	3,w6	1622 ± 171
D.H.	44	M	1,3	7,—	—,—	7,—	1808 ± 126
D.P.	54	M	2,3	7,40	(w3),—	5,w6	1528 ± 242
D.P.	46	F	1,w24	8,w39	—,—	3,—	1515 ± 116
J.R.	30	F	2,w24	14,w22	w3,—	1,2	1664 ± 163
Mean ± SD	41.6 ± 7.8						1642 ± 333

* CBMW, capillary basement membrane width.

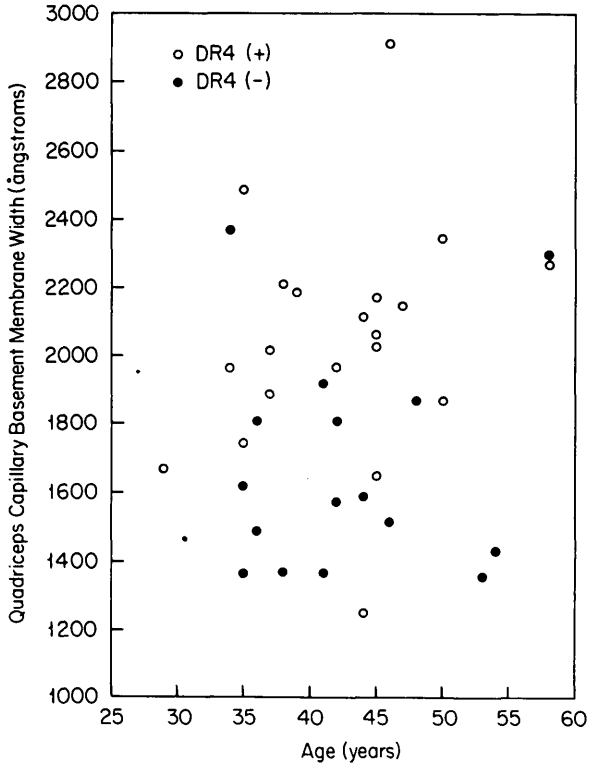

FIGURE 1. Relationship between age and quadriceps basement membrane width in parents of children with type I diabetes mellitus.

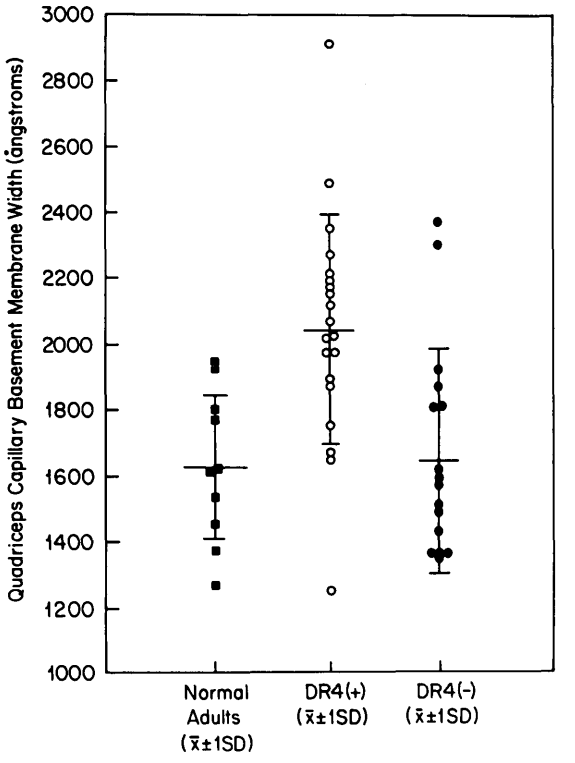

FIGURE 2. Quadriceps muscle capillary basement membrane width in DR4 positive (O) and DR4 negative (●) parents of children with type I diabetes mellitus.

tween the DR4 positive and DR4 negative parents was confirmed and shown to be independent of the age of the parents by analysis of covariance, using the method of Zar[22] (P = 0.0014). Analysis of the relationship of the capillary basement membrane width with age in the DR4 positive parents showed Pearson-R to be 0.287, P = 0.219. The same analysis of relationship of capillary basement membrane width to age in DR4 negative parents showed Pearson-R to be 0.209, P = 0.406. When the data for DR4 positive and DR4 negative parents were combined, no significant relationship between age and basement membrane width could be found (Pearson-R = 0.233, P = 0.159). These analyses were confirmed to be nonsignificant by calculation of Spearman's ρ and Kendall's τ as well. Thus no significant relationships could be established between age and membrane width in these subjects (Figure 2).

DISCUSSION

The results of this study show that a significant number of the parents of type I diabetic children, who themselves did not have diabetes, had thickened quadriceps capillary basement membranes, and that increased width of the capillary basement membrane correlated with the presence of HLA-DR4.

TABLE 5
Correlation between capillary basement membrane width and HLA-DR4

	CBMW*	
HLA phenotype of parents	> 2000 Å	< 2000 Å
HLA-DR4 positive	12	8
HLA-DR4 negative	2	16

* CBMW, capillary basement membrane width.
P(Fisher) < 0.005.

In a recent study by Deckert,[24] normal subjects had capillary basement membrane width of 1630 ± 330 Å (mean ± 1 SD) which compares closely with our data (1628 ± 218 Å). Thus, from previous results using the Siperstein technique at this institution[12,13,16] and in other studies,[24,25] it is apparent that capillary basement membrane values over 2000 Å are most unusual in normal subjects.

With two exceptions, the capillary basement membrane widths in affected offspring with type I diabetes were less than 2000 Å. Both subjects were greater than 18 yr of age at the time of study. These results are similar to those obtained previously with the same technique.[16] Capillary basement membrane widths were normal in the majority of children with type I diabetes also by another method which produces lower absolute values.[23] Fourteen of the 38 parents (37%) in the present study had capillary basement membranes greater than 2000 Å.

The factors leading to development of thickened capillary basement membranes, the mechanism causing the change, and its significance in relation to the vascular complications of diabetes are not well understood.[12–16,26] In an early study, Siperstein and co-workers[12,13] suggested that capillary basement membrane thickening can occur in the absence of overt metabolic abnormalities of diabetes. In children of diabetic parents who had not developed clinical evidence of the disease, thickening of capillary basement membranes was found in approximately one-half of the cases.[12] In the present report, we have found increased width of capillary basement membranes in nondiabetic parents of diabetic children. An age-related factor causing increased capillary basement membrane width has been previously observed[16] and is apparent in the present data from the nar-

row width of capillary basement membrane in children even in the presence of clinical diabetes. Capillary basement membrane thickness is unusual in prepubertal children.[16,23] However, as previously noted by others,[24] there was little effect of age on capillary basement membrane width within the age group of the parents. Analysis of covariance showed that the difference in capillary basement membrane width between the DR4(+) and DR4(−) parents was not related to age.

The three parents who had type I diabetes were excluded from the study. Because the remaining parents did not have any evidence of diabetes, other factors suggested as possible causes of capillary basement membrane thickening in diabetes[15,16] need not be considered.

The excess of DR4 among parents with thick capillary basement membranes is the most interesting finding in this study. Capillary basement membrane width was significantly greater in DR4 positive parents and slightly decreased in relation to DR3. Thus it appears that increased width of capillary basement membranes in parents of type I diabetics may depend, in part, on a genetic factor that is associated with HLA-DR4. This factor may somehow influence the activities of endothelial cells which appear to be responsible for the production of capillary basement membranes.[27] It is not known, however, whether the increased width of capillary basement membranes in diabetes is due to increased production or reduced turnover of basement membrane material.[28] It is also not known whether the mechanism(s) causing increase in capillary basement membrane width in diabetic patients and in nondiabetic relatives is (are) the same.

Finally, the relationship between DR4, increased capillary basement membrane width, and risk for development of diabetes is, at present, a mystery. Whether the DR4 associated factor that causes thickening of capillary basement membranes and the one that produces increased risk for development of diabetes are the same or different is not known. Evidently, increased risk due to the DR4-related genes occurs long before the capillary basement membranes have increased in width, by currently available techniques of measurement. It is possible, however, that abnormalities in the function of endothelial cells, and perhaps of other cells, exist long before a widening of the capillary basement membrane becomes evident.

It is possible that the abnormality detected in the capillary basement membranes of muscles in diabetic patients may be related to the microvascular changes that cause the retinal or glomerular lesions of diabetes. However, the evidence for a correlation between quadriceps muscle basement membrane width and vascular lesions in the eyes and kidneys is at present not very good[25] and severe progressive vascular lesions do not develop in the absence of hyperglycemia except in very rare instances.[29,30] Further studies will be required to determine the significance of the increased width of capillary basement membranes in subjects who are otherwise normal, as in the case of the parents in the present study. It is conceivable that such persons would more readily develop microvascular disease (e.g., retinopathy) in the presence of hyperglycemia.

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Note added in proof. Following the preparation of the manuscript, an abstract by Dornan et al. was published which suggested a relationship between HLA-DR4 and proliferative retinopathy in type I diabetics of long standing.[31]

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