Discordance of Diabetic Microangiopathy in Identical Twins


SUMMARY

In a pair of 19-year-old monozygotic twin girls, one developed insulin-dependent, ketosis-prone diabetes at the age of three and has required insulin for the past 16 years. Her identical twin has maintained normal oral and intravenous glucose tolerance with normal insulin release and glucagon suppression. An unequivocal hypertrophy of the muscle capillary basement membrane (1,800 ± 148 Å) was found in the diabetic twin, while a normal thickness of 1,149 ± 62 Å was present in her nondiabetic sister. Follow-up of the present subjects and data from other discordant identical twins who have reached adulthood could determine whether muscle capillary basement membrane hypertrophy is an independent marker of genetic diabetes in adults.

Discordance of diabetic microangiopathy in a pair of monozygotic twins has important implications regarding the influence of heredity and environment on diabetic microangiopathy. DIABETES 25:24-28, January, 1976.

The search for a specific and reliable genetic marker for diabetes mellitus remains a continuing challenge. Characteristic hormonal derangements are not necessarily consistent in all patients with diabetes and are much less so in those individuals not yet affected but who have a high likelihood of subsequently developing overt diabetes. In these suspected "prediabetics," deficient early insulin release after intravenous glucose has been a frequent, but not constant, abnormality, as has impaired suppressibility of growth hormone and glucagon after glucose loading. A much more consistent defect is the thickening of the basement membrane of muscle capillaries, reported to occur in 98 per cent of diabetic adults with fasting hyperglycemia of 140 mg. per deciliter or higher. Moreover, abnormal basement membranes were demonstrated in as many as 73 per cent of persons whose parents had diabetes mellitus but who themselves had normal carbohydrate tolerance. For these reasons, capillary basement membrane hypertrophy has been considered a likely genetic marker for diabetes mellitus. These findings have now been confirmed by a number of laboratories. However, Kilo et al. and Yodaiken et al. have reported a lower prevalence of basement membrane thickening in diabetic subjects than have the other investigators, but this discrepancy is probably explained by differences in the fixation and morphometric techniques used.

The present study, involving a pair of 19-year-old female monozygotic twins in only one of whom insulin-dependent, ketosis-prone juvenile diabetes developed at the age of three, provides a unique opportunity to investigate the possibility that capillary basement membrane thickness is predominantly genetically determined and represents an independent marker of genetic diabetes mellitus in adults. To our knowledge, this has not previously been studied in diabetic twins.

SUBJECTS

Twin girls, N.S. and D.S., were born on January 18, 1956. According to the parents, there was only one placenta, but no information is available as to whether this was monochorionic. At age three, N.S. spontaneously developed diabetes mellitus and has required daily insulin injections for the past 16 years. Her identical twin, D.S., has remained nondiabetic over this same period. As young children they looked identical, and this persisted until age 15, when N.S. acquired at least 18 to 22 lb. (8-10 kg.) more body weight, resulting in a rounding of her body contours and consequent distinctiveness from her slimmer sister. However, extensive studies in the University of
California Genetics Clinic have established their identicalness with a 99.1 per cent probability based on the identity of five distinctive blood groups, polymorphisms, dermatoglyphics, and histocompatibility antigens (HLA-A3, HLA-AW26, and HLA-B27).

At age six, both twins developed a relatively severe case of mumps as documented by their father, a pediatrician. No other serious or unusual childhood diseases occurred, and neither twin has become pregnant. Recently, their mother, aged 47 and nonobese, developed hyperglycemia and glycosuria while taking glucocorticoid therapy for chronic asthma. This diabetic state has been mild and controllable with diet and, occasionally, insulin. Although there is a history of mild adult-onset diabetes in a paternal uncle, three other siblings (ages 17, 22, and 24) and their father have no history of diabetes.

N.S. This twin always weighed more than her sister, being 6 lb. 4 oz. at birth as against D.S.’s weight of 5 lb. 10 oz., and was slightly taller and heavier at age three, although both were in the normal range for weight at the time N.S. developed diabetes. There was no obvious precipitating event at the onset of her diabetes, nor has there been a past history of pancreatitis. Control of her diabetes has been less than ideal: postprandial glycosuria averages 2 to 3+ on a daily mixture of 50-60 U. of NPH and regular insulin given in two divided doses. Trace proteinuria has been observed on occasion since 1974, with normal creatinine clearance of 90 ml. per minute. Blood pressure has remained at 120/70 mm. Hg. Mild “background” diabetic retinopathy was observed, with three or four microaneurysms noted in each fundus. Her present height is 5 feet 2 inches (154 cm.) and her average weight is 132 lb. (60 kg.), 16 per cent over ideal weight.

D.S. This twin has never had symptoms or signs of diabetes mellitus and has always been in good health except for the usual childhood diseases, including, as noted above, mumps at age six along with her sister. Although smaller than N.S. for most of her childhood, she recently passed her slightly in height, being 5 feet 4 inches (159 cm.) tall, yet she manages by careful dieting to remain at a lesser weight of 112 lb. (50 kg.).

METHODS

Blood groups, haptoglobin, glucose-6-phosphate dehydrogenase, transferrin, and group-specific component were assessed in venous blood samples from both parents, two siblings, and both twins by methods reviewed by Brock and Mayo. Dermatoglyphics were assessed by direct counting of finger ridges over a comparable surface of all 10 fingers in each twin.

Oral and intravenous glucose tolerance tests were carried out on the nondiabetic twin after an overnight fast and after three days on a 200-to-300-gm. carbohydrate diet. (For the former, 100 gm. of glucose in 250 ml. of water was used; for the latter, 25 gm. of glucose was infused as a 50 per cent solution over three minutes as previously described.) Plasma glucose was measured by the Beckman glucose oxidase method, and serum insulin, growth hormone, and plasma glucagon levels were measured by immunoassay as previously described. Antimicrosomal and antithyroglobulin thyroid antibodies were measured by quantitative hemagglutination. The technic for obtaining quadriceps muscle biopsy specimens (fixation procedure with osmic acid) and the method of quantifying mean capillary basement membrane thickness have been previously reported.

RESULTS

Assessment of Monozygosity (Table 1)

Although all 11 blood groups and polymorphisms studied in the twins were identical, only five of these were informative for zygosity determination in that they were variant in the parents. The twins were concordant for all five factors (CDe, N, k, haptoglobin type 2-2, and group-specific components type 1-1). Total dermal finger-ridge counts were 98 for N.S. and 99 for D.S. From the formula of Bulmer, a probabil-

**TABLE 1**

Factors governing determination of monozygosity in D.S. and N.S.

<table>
<thead>
<tr>
<th>Relative probability of event's occurring</th>
<th>if monozygous</th>
<th>if dizygous</th>
</tr>
</thead>
<tbody>
<tr>
<td>A priori from population</td>
<td>0.004</td>
<td>0.008</td>
</tr>
<tr>
<td>Same sex</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Same CDe</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Same N</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Same k</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Same type haptoglobin</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Same type group-specific component</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Ridge-count difference = 1</td>
<td>0.0249</td>
<td>0.00753</td>
</tr>
</tbody>
</table>

Product of above probabilities: $9.95 \times 10^{-5}$ $9.41 \times 10^{-7}$

Probability of monozygosity: $9.95 \times 10^{-6} = 0.991$

*Method of Bulmer"
ity of 99.1 per cent for monozygosity can be derived from the concordance of these factors and the ridge-count difference of one.

ENDOCRINE STUDIES

N.S. (Table 2)

Fasting hyperglycemia occurred frequently in this patient and was associated with inappropriately elevated plasma glucagon concentrations during four of the five times they were measured. This is in contrast with the normal glucagon suppressibility manifested in her nondiabetic twin, in whom plasma glucagon levels fell to 10 and 13 pg. per milliliter with plasma glucose concentrations of 121 and 152 mg. per 100 ml. during glucose administration (table 3). Insulin measurements could not be performed because of the presence of insulin antibodies after 16 years’ administration of exogenous insulin, which interferes with estimation of endogenous insulin by standard immunoassay.

Serum thyroxine, triiodothyronine resin uptake, and free thyroxine index were normal. Antimicrosomal thyroid antibodies were positive in a dilution of 1:1,600, but antithyroglobulin antibodies were undetectable. Normal subjects have antimicrosomal titers of less than 50, whereas titers from 100 to 25,600 are seen with this method in 73 per cent of patients with Hashimoto’s thyroiditis. However, in 95 per cent of these cases antithyroglobulin antibodies were usually positive.

D.S. (Table 3)

Oral and intravenous glucose tolerance values in D.S. were clearly not diabetic, according to American Diabetes Association criteria for oral testing and the criteria of Lundbaek for intravenous glucose tolerance.

Hormonal responses to oral glucose. Fasting insulin levels were within the normal range (mean in 12 normal subjects: 11 μU. per milliliter; range: < 5 to 25 μU. per milliliter). There was no delay or impairment in insulin release, and maximum rise was well within normal limits (peak less than 120 μU. per milliliter at one hour and less than 100 μU. per milliliter at two hours). Plasma glucagon showed a basal level well within the normal range (mean ± S.E.M.: 63 ± 19 pg. per milliliter) and was promptly suppressed in normal fashion after ingestion of glucose.

Hormonal responses to intravenous glucose. Rapid early insulin release occurred in a normal pattern, reaching 107 μU. per milliliter within one minute after ending a two-minute infusion of 25 gm. of glucose. The disappearance rate of glucose (Kg) at 2 per cent per minute and the rapid suppression of plasma glucagon after 30 minutes are further indications of normal metabolic responsiveness.

Thyroid status. Serum thyroxine, triiodothyronine resin uptake, and free thyroxine index were normal. Antimicrosomal thyroid antibodies were positive in a dilution of 1:100, whereas antithyroglobulin antibodies were undetectable.

MUSCLE CAPILLARY BASEMENT MEMBRANE MORPHOMETRY (TABLE 4)

Measurements of 15 capillaries from a quadriceps muscle biopsy showed an average basement membrane thickness of 1,800 ± 148 Å (mean ± S.E.M.) in N.S., which is well above the cut-off point of 1,600 Å or over—a value exceeded by no more than 1 per cent of normal control subjects and at least 93 per cent of diabetics aged 19 or older.

The mean thickness in D.S. was only 1,149 ± 62 Å, a value clearly in the center of the normal range.
TABLE 4

Average muscle capillary basement membrane width in the diabetic and the nondiabetic twin

<table>
<thead>
<tr>
<th></th>
<th>Mean capillary basement membrane width (Mean ± S.E.M.)</th>
<th>Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>N.S. (diabetic)</td>
<td>1,800 ± 148</td>
<td>1,373</td>
</tr>
<tr>
<td>D.S. (nondiabetic)</td>
<td>1,149 ± 62</td>
<td>1,373</td>
</tr>
<tr>
<td>Normal subjects (N = 50)</td>
<td>1,080 ± 27*</td>
<td>1,373</td>
</tr>
<tr>
<td>Diabetic adults (N = 75)</td>
<td>2,364 ± 88†</td>
<td>1,149</td>
</tr>
<tr>
<td>Prediabetics (N = 30)</td>
<td>1,373 ± 44</td>
<td>1,080</td>
</tr>
</tbody>
</table>

*Normal upper limit = 1,325 Å.
†Diabetic range = 1,600 and over.

(J ninety-nine per cent of diabetics have measurements higher than 1,325 Å.)

DISCUSSION

There seems to be little question that these twins, discordant for diabetes mellitus as well as for diabetic microangiopathy, are truly monozygotic (based on their similar appearance and the genetic data described above). The unique feature of this report is the unequivocal demonstration of hypertrophy of muscle capillary basement membranes in the diabetic member of a pair of identical twins and normal capillary basement membranes in the nondiabetic twin. Siperstein reports evidence from his own work and that of others that this lesion represents an independent marker of genetically determined diabetes mellitus. His conclusions are based on the almost universal presence of this defect in adults with diabetes mellitus as well as in 73 per cent of persons with normal carbohydrate tolerance whose parents were both diabetic. The specificity of capillary basement membrane thickening as a marker of genetic diabetes mellitus is supported by the observation that patients with hyperglycemia secondary to pancreatitis, Cushing's syndrome, muscular dystrophy, myotonia dystrophica, and hyperlipidemia (even of long duration) rarely develop this lesion.

However, if thickening of the capillary basement membranes in N.S. indicates a genetic basis for her diabetes, one might have expected her identical twin to show this defect too, although this may be a function of her age. Raskin et al. showed that, even in overt diabetics under age 20, only 40 per cent had abnormally thickened basement membranes. The prevalence of basement membrane thickening would presumably be even less in genetically prediabetic subjects in this age group. Since these twins are 19 years old, they may still be in the borderline age group and should be re-examined in several years before assuming that the unaffected twin's definitely normal basement membranes will not thicken as she achieves full adulthood.

Other conceivable explanations for genetic diabetes manifested by capillary basement membrane hypertrophy in one only of a pair of monozygotic twins include the less likely possibilities of a unique mosaicism carrying the diabetic gene or a somatic mutation during early development.

Alternatively, it remains possible that N.S. has a nongenetic, acquired form of diabetes and that her capillary basement membrane thickening has resulted from the length of time during which her hyperglycemia was only under fair control. Until more data become available on the effect of acquired diabetes for as long as 16 years on capillary basement membrane thickening, this possibility cannot be excluded. The long-held assumption that the nonaffected identical twin of a diabetic was necessarily a prediabetic has been reviewed. Current evidence based on epidemiologic studies of as many as 272 pairs of identical twins, one or both of whom had diabetes, suggests that a genetic component may be a less important factor in those twins who developed diabetes before the age of 40 than in those who developed it after. Tattersall and Pyke show that, when one identical twin develops diabetes before age 10 (as was the case in our patient), only 61 per cent of the unaffected twins become diabetic. Moreover, in those twins who remain unaffected for as long as 10 years, these authors foresee little likelihood that diabetes will develop. This observation is further supported by the finding that, in identical twins who have been discordant for diabetes for at least 10 years, the unaffected twin's normal glucose tolerance seldom deteriorates with time and both insulin release and glucagon suppression after glucose are indistinguishable from normal. Thus, the present case of diabetes mellitus of juvenile onset in one of a pair of identical twins with no evidence of diabetes in the other twin despite a 16-year time interval would suggest an environmental, rather than a genetic, basis for the diabetes in the affected twin. The demonstration of a positive titer of antimitosomal thyroid antibodies may be relevant in implicating autoimmune mechanisms as a factor in the development of her diabetes. If a nongenetic type of diabetes is implicated in N.S. by further long-term follow-up of her unaffected twin, it would support the thesis that capillary microangiopathy may be acquired consequent to long-standing hyperglycemia or...
other metabolic effects of inadequate insulin replacement or both.

Regardless of the exact mechanism for this phenomenon, the demonstration of discordance of diabetic microangiopathy in a pair of monozygotic twins has implications regarding the influence of heredity versus environment on microangiopathy. Long-term follow-up of the present subjects and data from other discordant diabetic twins would be relevant in answering this question.

ACKNOWLEDGMENTS

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REFERENCES