HLA-DR typing in identical twins with insulin-dependent diabetes: difference between concordant and discordant pairs

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Abstract

A total of 106 pairs of identical twins, of whom 56 were concordant and 50 discordant for insulin-dependent diabetes, were typed for HLA-DR. In both the concordant and discordant groups there was a high prevalence of the antigens DR3 and DR4, a low prevalence of DR5 and DR7, and a virtual absence of DR2. The heterozygous phenotype DR3,DR4 was more prevalent in concordant than discordant pairs. This was therefore the first demonstration of a genetic difference between concordant and discordant identical twin pairs.

These findings suggest that possession of both DR3 and DR4 antigens confers a greater genetic predisposition to insulin-dependent diabetes than does the possession of either antigen alone.

Introduction

In our study of diabetes in identical twins at King's College Hospital the rate of concordance (both twins diabetic) for insulin-dependent diabetes is roughly 50%.[1] This is a much higher prevalence of concordance than recorded from series of non-identical twins[2] and suggests that genetic factors are important in the development of the disease.

An association between insulin-dependent diabetes and the HLA complex has been described. Initially the strongest association was with the B locus and the antigens B8 and B15.[3 4] It was therefore suggested that gene(s) associated with the B locus were important in the disease. A stronger association was shown for the heterozygous phenotype B8,B15, suggesting that two independent genes were important.[4 5] When the prevalence of the HLA-B antigens was studied in a series of insulin-dependent identical twin pairs the association with B8 and B15 was confirmed.[6] Furthermore, the prevalence of both antigens was increased in concordant as well as discordant twin pairs.[7] These results suggested that the same genetic factors might be operating in both groups of twins and that differences in the prevalence of HLA-B antigens did not explain the difference between concordance and discordance. There was, however, a trend towards an increase in the heterozygote B8,B15 in the concordant pairs.

The association between insulin-dependent diabetes and the B locus is now known to be secondary, the primary association being with the DR locus. The increase in prevalence of B8 is due to linkage disequilibrium with DR3, B15 with DR4 in probands with the disease. An increase in the prevalence of the heterozygote DR3,DR4 has been noted in many series and in white people is associated with the highest known relative risk for insulin-dependent diabetes.[8-12] This suggests that the possession of both antigens confers a greater genetic predisposition to insulin-dependent diabetes than does the possession of either antigen alone.

We have investigated the HLA-DR phenotypes in identical twins with insulin-dependent diabetes. We wished to see whether we could confirm the association with HLA-DR3 and DR4 and whether there was any difference in the prevalence of particular DR phenotypes between concordant and discordant twin pairs.

Patients and methods

We tested 106 pairs of twins, of whom 56 were concordant and 50 discordant. Their ages ranged from 5 to 79 years. All diabetics were truly insulin dependent. All were white and living in the United Kingdom. The unaffected twins of discordant pairs were asymptomatic. All had had a normal response in a 50 g oral glucose tolerance test and at the time of study had normal random blood glucose and haemoglobin A_1 concentrations. To establish as far as possible that the discordant pairs were truly discordant we included them only if at least five years had elapsed since the affected twin had been diagnosed. Proof of identity was established as described.[13] Controls were selected from medical, technical, and nursing staff at St Bartholomew's Hospital and husbands and wives attending the antenatal clinic. All were white and none had a family history of diabetes or other autoimmune disease.

HLA-DR typing—Whole blood (10 ml) was collected in 8 ml tissue culture medium 199 (Wellcome) with the addition of 2 ml sodium citrate 3·8%. After collection the samples were kept airtight at room temperature and analysed within 24 hours. DR typing was performed by a two-colour fluorescence technique.[14] All 10 officially recognised DR specificities were defined by using a minimum of 60 antisera. Statistical analysis was by χ^2 test with Yates's correction and Fisher's exact test.

Results

The twin population showed an increase in prevalence of the antigens DR3 and DR4 and a decrease in DR2, DR5, and DR7 (table I). Only five twin pairs had neither of the antigens DR3 and DR4.

Both the concordant and discordant twins showed an increased prevalence of DR3 and DR4 as compared with the controls (table II). There was, however, no significant difference in the prevalence of these antigens between concordant and discordant twins. Nevertheless, there was a difference in the prevalence of the antigen combinations in the concordant and discordant pairs. More of the concordant than

TABLE I—*Frequency of HLA-DR antigens in identical twins and controls*

DR antigens	Twins			Controls (n = 110)	χ^2 (difference between twin population and controls)
	Concordant (n = 56)	Discordant (n = 50)	Total (n = 106)		
DR1	9	7	16	20	NS
DR2	1	0	1	31	29·6
DR3	41	27	68	35	21·4
DR4	47	33	80	37	36·4
DR5	3	1	4	19	9·0
DRW6	3	10	13	14	NS
DR7	0	8	8	39	23·0
DRW8	1	1	2	3	NS
DRW9	0	2	2	3	NS
DRW10	1	1	1	2	NS
DR Blank*	6	10	16	17	NS

NS = Not significant.
* DR Blank symbolises either homozygous phenotype for one of the antigens tested or antigen as yet unidentified.

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of the discordant pairs were heterozygote DR3,DR4—33 (59%) versus 14 (28%) ($\chi^2=9{\cdot}02$; $p<0{\cdot}01$) (table III). This increase in the heterozygote DR3,DR4 was seen in the concordant pairs at all ages of onset of diabetes, being found in 12 out of 21 (57%) of the concordant twin pairs in whom the proband—that is, the affected twin in the discordant pairs and the first diagnosed in the concordant pairs—was diagnosed under the age of 10 and 12 out of 21 (57%) from 10 to 20 years and in nine out of 14 (64%) of those pairs diagnosed over the age of 20.

TABLE II—*Numbers of concordant and discordant twins possessing antigens DR3 and DR4*

	Concordant (n = 56)	Discordant (n = 50)	Controls (n = 110)
DR3	41 ($\chi^2=24{\cdot}0$; $p<0{\cdot}001$)*	27 ($\chi^2=6{\cdot}2$; $p<0{\cdot}02$)*	35
DR4	47 ($\chi^2=35{\cdot}6$; $p<0{\cdot}001$)*	33 ($\chi^2=13{\cdot}3$; $p<0{\cdot}001$)*	37

* Comparison of prevalence of antigen with control population.

TABLE III—*HLA-DR phenotypes of twin pairs. Figures are number (%) of twin pairs*

	DR3,DR4	DR4,X*	DR3,X*	X,X*
Concordant (n = 56)	33 (59)	14 (25)	8 (14)	1 (2)
Discordant (n = 50)	14 (28)	19 (38)	13 (26)	4 (8)
Significance	$p<0{\cdot}01$	NS	NS	NS
Controls (n = 110)	7 (6)			

NS = Not significant.
* X symbolises one of the following DR antigens: DR1, DR2, DR5, DRW6, DR7, DRW8, DRW9, DRW10, DR Blank.

Most of the concordant twin pairs became concordant within five years. The time to become concordant, however, was unrelated to the DR phenotype, the heterozygote DR3,DR4 being as common in the twins becoming concordant within five years as in those becoming concordant after a longer period—within five years, 19 out of 35; more than five years, 14 out of 21.

We did not study the families of the twins and therefore could not identify DR3 or DR4 homozygosity, since it is impossible to exclude the possibility of an antigen as yet undetected. If we assume that all those twin pairs in whom we could not detect an antigen other than DR3 or DR4 were homozygous for these antigens then three concordant and four discordant pairs were homozygous for DR3, and three concordant and six discordant pairs were homozygous for DR4.

The twin series as a whole compared with the controls showed a reduced prevalence of DR2, DR5, and DR7 (table I). DR2 was virtually absent, appearing in only one twin pair; DR7 was found in none of the concordant twins but in eight of the discordant pairs. This difference was significant ($p=0{\cdot}00178$; Fisher's exact test).

Age—The only other detectable difference between twin pairs was in the age at onset in the probands: among the concordant pairs 42 were diagnosed before the age of 20 and 14 over 20, and among the discordant pairs 28 were diagnosed before the age of 20 and 22 over 20 ($\chi^2=4{\cdot}25$; $p<0{\cdot}05$). Thus the ratio of concordance to discordance in pairs in which the proband was diagnosed under the age of 20 (3:2) was higher than in older pairs (2:3). Identical ratios were observed when the twin pairs were divided into those living together (3:2) and apart (2:3) at the time of diagnosis of the proband.

Discussion

These insulin-dependent diabetic twins, both concordant and discordant, showed a high prevalence of HLA-DR3 and DR4 when compared with a non-diabetic control population, suggesting that similar genetic factors were operating in both groups. There was, however, a striking difference in the prevalence of the heterozygote DR3,DR4 between the two groups, being twice as common in the concordant as in the discordant pairs. This is the first genetic difference between the two groups of twins that we have been able to identify. When the prevalence of the heterozygote DR3,DR4 was compared with that in other series of insulin-dependent diabetics the concordant pairs showed a higher and the discordant pairs a lower prevalence. The figure in discordant pairs lay about halfway between the control population and insulin-dependent diabetics.[9-12] These results suggest that the possession of both DR3 and DR4 confers a greater genetic susceptibility than does the possession of either antigen alone. This is supported by studies on families with more than one affected sibling in which the other siblings were more likely to be affected if they had both these antigens.[15]

There are two possible explanations for the reduced prevalence of HLA-DR2, DR5, and DR7: (*a*) these antigens may have a primary role in protecting against insulin-dependent diabetes, and (*b*) the reduction may have been secondary, as a result of the increase in DR3 and DR4. We have no way of distinguishing between these two possibilities but the virtual absence of DR2 in the concordant and discordant pairs, as in many other series of insulin-dependent diabetics[8 10 12] suggests that possession of this antigen may be protective. DR7 is also reduced in insulin-dependent diabetes,[8] and interestingly none of our concordant pairs possessed this antigen, though eight of the 50 discordant pairs did. This difference in prevalence is another genetically determined difference between concordant and discordant pairs and suggests that DR7 also may confer protection.

The concordant and discordant pairs also differed in their age at onset of diabetes. The increase in concordance seen in the younger twin pairs could not be attributed to the distribution of DR phenotypes: the heterozygote DR3,DR4 was more common in the concordant than discordant pairs irrespective of the age at onset of diabetes. The twins were also more likely to be concordant if they were living together at the time of diagnosis of diabetes in the proband; therefore, the fact that more of the younger pairs were concordant might have been because both twins shared a common environment. On the other hand, the increase might reflect a bias in ascertainment, since notification of twin pairs is more likely if they are younger and concordant. We do not, however, think that any bias in ascertainment would account for the differences in the DR phenotypes between the concordant and discordant twins.

We cannot be certain that discordant pairs are truly discordant, since an unaffected twin might still develop diabetes. Nevertheless, we believe that this is unlikely because (*a*) only pairs who had been discordant for at least five years were included, and in most concordant pairs the second twin develops diabetes within five years of the first[1]; and, (*b*) none of the unaffected twins showed any chemical evidence of diabetes.

This study shows that identical twins are not a homogeneous population and that there are detectable genetic differences between concordant and discordant pairs. As a result, analyses using the concordance rate in identical twins as an indication of gene penetrance must be interpreted with caution. The heterozygous phenotype DR3,DR4 appears to confer a greater genetic susceptibility to insulin-dependent diabetes than either antigen alone, which strongly favours the theory that the disease is associated with at least two independent genes closely linked to the HLA-DR locus.

If only one susceptibility gene linked to the DR locus is important for development of the disease then homozygosity for either DR3 or DR4 should confer a greater genetic predisposition. We studied phenotypes not genotypes and therefore were unable to identify with certainty the presence of homozygosity. The blanks in table I may represent a DR antigen as yet unrecognised. If, however, we assume that they do indicate a homozygous form then there was no increase in either homozygous DR3 or DR4 in the concordant as against the discordant pairs. Furthermore, family studies show that homozygosity for DR3 or DR4 does not confer an increased susceptibility to the development of insulin-dependent diabetes.[9 12 16]

We conclude that our data derived from the twins together with the family studies are incompatible with a single-gene theory for the development of insulin-dependent diabetes.

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Long-term continuous intraperitoneal insulin treatment in brittle diabetes

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Abstract

Attempts to achieve a fair metabolic equilibrium in a young woman with brittle diabetes by continuous subcutaneous, intramuscular, and continuous intravenous administration of insulin were unsuccessful. Continuous intraperitoneal administration of insulin through a permanently inserted polyethylene catheter connected to an open-loop peristaltic pump led to an appreciable improvement in mean blood glucose concentration, mean amplitude of glycaemic excursions, and M value and to normalisation of intermediate metabolic products. The peritoneal catheter was well tolerated for over 120 days without appreciable adverse effects.

This case suggests that long-term intraperitoneal administration of insulin is a feasible therapeutic approach in the management of brittle diabetes.

Introduction

Brittle diabetes is a heterogeneous clinical condition characterised by unpredictable swings in blood glucose concentration, an increased number of admissions to hospital, and changes in daily insulin requirement sometimes associated with altered or irregular insulin absorption.[1] We report here attempts to control severe brittle diabetes in a young woman: continuous subcutaneous, intramuscular, or continuous intravenous administration of insulin was unsuccessful, but use of a permanent intraperitoneal catheter connected to a peristaltic pump resulted in fair metabolic equilibrium.

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Case report

The patient, a 28 year old schoolmistress who was slightly overweight, had been diagnosed as diabetic in 1967, when she was 14, since when she had been receiving insulin treatment. After a year of relative equilibrium her diabetes had become unstable, with frequent episodes of ketosis; she had been admitted to hospital on at least 13 occasions over the past seven years because of hyperglycaemic coma and on numerous other occasions each year because of severe hypoglycaemia. Glycosylated haemoglobin concentration during the past year had been over 15%; C peptide was undetectable in serum even during severe hyperglycaemia. She was first admitted to the metabolic ward of this hospital in October 1980; she was then twice admitted to the emergency departments of two other hospitals during the following months with a diagnosis of diabetic ketoacidosis, and she was readmitted to our metabolic ward in December 1981.

During the following 130 days different routes of insulin administration were tried—namely, continuous subcutaneous, intramuscular, continuous intravenous, and, finally, since these were all unsatisfactory, continuous intraperitoneal (using a peristaltic pump, Promedos E1; Siemens, West Germany). An intraperitoneal, silicone-coated, high-density polyethylene catheter (internal diameter 0·3 mm, external diameter 0·7 mm; Siemens) was inserted percutaneously through a 14-gauge Teflon cannula (Abbocath 14) 4 cm below the umbilicus on the linea alba, a needle introducer being used to make a 15-cm subcutaneous tunnel: this technique reduces the amount of subcutaneous tissue dissected, establishes accurately the point of exit from subcutaneous tissue, and increases the stability of the catheter.