

No Evidence for Linkage Between HLA and Maturity Onset Type of Diabetes in Young People

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Summary. Studies with 20 different genetic marker systems were performed in a large kindred including 18 members affected with maturity onset type of diabetes of young people. Linkage closer than $0.1 \leq \theta$ could be excluded for ABO and

Gm, and linkage closer than $0.05 \leq \theta$ for HLA, GLO, and haptoglobin. No significant positive lod scores were found.

Key words: Linkage, HLA, MODY, genetics of diabetes, heterogeneity.

Within the last decade it has been increasingly clear that diabetes mellitus is a heterogeneous group of diseases; in 1973–1975, the final proof was obtained that Type 1 (insulin-dependent) diabetes and Type 2 (non-insulin-dependent) diabetes are genetically different: only Type 1 is associated with certain HLA types [1, 2, 3]. At the same time, Tattersall and Fajans [4] described a third type of diabetes: maturity onset type of diabetes of young people (MODY), with onset in adolescence, autosomal dominant inheritance, no or very little insulin requirement, and very few microangiopathic complications. Barbosa et al. [5] have studied HLA types in one family with MODY and found evidence for linkage although this was not statistically significant. We present here a linkage study with 20 different genetic markers, including HLA, in one large kindred with MODY.

Patients and Methods

Patients

The kindred studied here consists of 53 members and was investigated and reported in detail by Johansen and Gregersen [6]. The individual numbers in the pedigree (Fig. 1) are the same as the individual numbers in Figure 1 in that report. All family members except a large branch without affected members (descendants of individual no. 7) were asked to participate in blood sampling. Blood samples were obtained from 18 affected and eight unaffected family members as well as eight spouses. Individual no. 1 was diagnosed as diabetic at the age of 40 years in 1929; all others affected were diagnosed before the age of 20 years. Only one of the unaffected individuals (no. 39) is less than 20 years old.

Genetic markers

The following genetic markers were determined: HLA (see below); twelve serum protein and red cell enzyme markers – haptoglobin (Hp), group-specific component (Gc), immunoglobulin allotypes (Gm and Km), phosphoglucomutase, locus 1 (PGM₁), acid phosphatase (AcP₁), adenylate kinase (AK₁), 6-phosphogluconatedehydrogenase (PGD), glutamate pyruvate transaminase (GPT), carboxylesterase D (EsD), glutamate oxalate transaminase (GOT₁) and glyoxalase I (GLO); and seven blood group systems – ABO, Rhesus, Duffy, Kell, P, Lewis and Lutheran. The different genetic markers can be assigned to the following chromosomes according to the International System for Human Gene Nomenclature (1979) [7] (chromosome numbers in parentheses): HLA (6), Hp (16), Gc (4), GM (inconsistent 6 or 7), KM (7), PGM₁ (1), AcP₁ (2), AK₁ (9), PGD (1), GPT (unknown), EsD (13), GOT₁ (10), GLO (16), ABO (9), Rhesus (1), Duffy (1), Kell (unknown), P (6), Lewis (unknown), and Lutheran (unknown).

HLA typing

HLA typing for the HLA-A, B, and C antigens was performed as described previously [8]. We typed for the following specificities (splittings are in parentheses): From the A series: A1, A2, A3, A9 (Aw23, Aw24), A10 (A25, A26), A11, A28, and Aw19 (A29, Aw30, Aw31, Aw32). From the B series: B5, B7, B8, B12 (Bw44, Bw45), B13, B14, B15, Bw16 (Bw38, Bw39), B17, B18, Bw21 (Bw49, Bw50), Bw22 (Bw55, Bw56), B27, Bw35, B37, B40, Bw41, Bw47, and Bw53. From the C series: Cw1, Cw2, Cw3, Cw4, and Cw6. In this paper B12 equals Bw44.

Linkage analysis

Lod scores (z) were calculated with a slightly modified version of a previously described programme [9]. The main feature of this programme is the breaking down of each pedigree into a number of nuclear families consisting of a sibship with the parents and grandparents. These nuclear families are then analyzed separately, the lod scores being finally

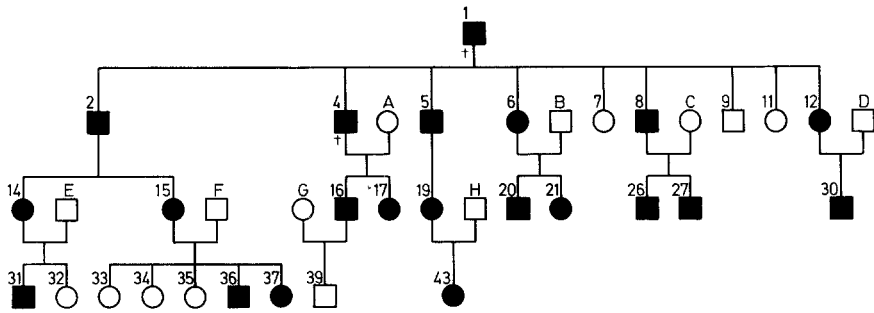


Fig. 1. Pedigree of MODY family. Individual numbers refer to individual numbers in Figure 1 in reference no. 6. Filled symbols denote members affected with MODY

Table 1. HLA genotypes in MODY family

Individual number (see Fig. 1)	Paternal/maternal haplotype HLA-A, C, B/ A, C, B
2	2, w3, 40/28, w3, 40
6	2, w3, 40/28, w3, 40
7	2, w3, 40/ 1, w4, w35
8	2, w3, 40/28, w3, 40
9	2, w3, 40/28, w3, 40
11	2, -, w38/ 8, w3, 40
12	2, -, w38/28, w3, 40
14	2, w3, 40/ , , ^a
15	2, w3, 40/ 1, -, 8
16	28, w3, 40/ 1, -, 12
17	28, w3, 40/w32, w2, 40
19	1, w4, w35/2, -, 7
20	1, -, 37/ 2, w3, 40
21	1, -, 37/28, w3, 40
26	2, w3, 40/28, w4, w35
27	2, w3, 40/w24, w4, w35
30	28, -, 14/28, w3, 40
31	w19, -, 7/ 2, w3, 40
32	w19, -, 7/ 2, w3, 40
33	28, w2, 27/ 2, w3, 40
34	1, -, 8/ 2, w3, 40
35	1, -, 8/ 1, -, 8
36	1, -, 8/ 2, w3, 40
37	28, w2, 27/ 1, -, 8
39	28, w3, 40/24, w3, 15
43	1, -, 8/ 2, -, 7
spouses	
A	1, -, 12/w32, w2, 40
B	1, -, 37/ 3, -, 14
C	w24, w4, w35/28, w4, w35
D	28, -, 14/ 3, w4, w35
E	w19, -, 7/ 2, -, w50
F	28, w2, 27/ 1, -, 8
G	24, w3, 15/11, w4, 15
H	1, -, 8/28, w3, 40

^a Individual no. 14 can on the maternal haplotype be either A2, Cw3, B40 or blank. - Denotes blank

summed over all nuclear families belonging to a pedigree. Lod scores were calculated for a standard set of recombination fractions (θ), i.e. 0.05, 0.10, 0.20, 0.30, and 0.40. For each fraction, lod scores dependent on recombination in the fathers (z_m) were kept separate from lod scores dependent on the mothers (z_f).

In contrast to previous versions, the programme now also analyses nuclear families in which neither of the parents (nor grandparents) were tested for the marker and where deduction of their genotypes was not possible. This is done by considering all possible genotypes, weighted with respect to their population frequencies as calculated from gene (or haplotype) frequencies available from population studies.

Due to limitations in the available computer storage capacity (CDC CYBER 173 system), the programme can maximally consider ten haplotypes in a given genetic system. This is sufficient for nearly all systems, even Rhesus, but not for HLA. In this case a simplified model with eight codominant haplotypes, A, B, C, ... H of equal frequencies (i.e. 0.12375), and a rare null haplotype with a one percent frequency was adopted. The family members were then coded according to their deduced haplotypes which were labelled A, B, C ... H within each nuclear family. HLA haplotypes were deduced using all available typing data in the family, as apparent from Figure 1 and Table 1.

Results

Table 1 gives the HLA genotypes of all individuals in the pedigree (except for individuals nos. 1, 4, and 5; Fig. 1) and Table 2 gives the significant lod scores (by convention < -2.0 or > 3.0) found in this family. No significant positive linkage to MODY was found for any of the 20 investigated genetic marker systems. HLA-haplotypes appear in Table 1. By simple inspection, tight linkage between HLA and the 'MODY' gene(s) is unlikely. For example, in the three-generation transmission (individuals nos. 2, 15, and 37) no haplotypes are transmitted from individual no. 2 to no. 37. One pair of HLA-non-identical affected siblings is found and several HLA-identical individuals are discordant for the disease state.

Linkage analyses confirmed that close linkage between HLA and MODY can be excluded (z value of -2.7 for $\theta=0.05$). Linkage between MODY and ABO or Gm closer than between 10-20 centiMorgan, and for HLA, GLO, or Hp closer than between 5-10 centiMorgan, can be excluded.

Negative lod scores at $\theta=0.05$ of -1.6 , -1.4 , and -1.4 were obtained for Rhesus, GPT, and Gc, respectively. The remaining lod scores ranged between 0.002 and 0.255 at $\theta=0.05$.

Discussion

More than 30 distinct genetic disorders associated with carbohydrate intolerance have been described [for review 10]. Most of these syndromes are rare and collectively they comprise only a small fraction of all cases of diabetes. However, their existence illustrates the point that diabetes is just a syndrome which may cover several different aetiologies.

Table 2. Lod scores for genetic markers showing significant negative linkage with MODY

Recombination frequency θ	HLA			Glyoxalase 1			ABO blood group			Immunoglobulin allotype (Gm)			Haptoglobin (Hp)		
	Paternal	Maternal	Combined	Paternal	Maternal	Combined	Paternal	Maternal	Combined	Paternal	Maternal	Combined	Paternal	Maternal	Combined
0.05	-0.204	-2.443	-2.647	0.0	-2.442	-2.442	0.045	-3.794	-3.749	-0.295	-3.442	-3.837	0.079	-2.721	-2.642
0.10	-0.012	-1.587	-1.599	0.0	-1.586	-1.586	0.036	-2.597	-2.561	-0.174	-2.285	-2.459	0.064	-1.842	-1.776
0.20	0.073	-0.786	-0.713	0.0	-0.786	-0.786	0.020	-1.419	-1.399	-0.029	-1.184	-1.213	0.037	-0.990	-0.953
0.30	0.052	-0.373	-0.321	0.0	-0.373	-0.373	0.009	-0.754	-0.745	0.002	-0.595	-0.593	0.017	-0.520	-0.503
0.40	0.016	-0.132	-0.116	0.0	-0.132	-0.132	0.002	-0.311	-0.309	0.003	-0.229	-0.226	0.004	-0.212	-0.208

Lod scores are calculated for paternal and maternal as well as combined segregation at different recombination frequencies (θ).

Type 1 diabetes has now been clearly separated from Type 2 diabetes not only on the basis of the HLA association, but also on the basis of concordance rate in monozygotic twins in that less than 50% of Type 1 monozygotic twins are concordant for diabetes, while nearly all Type 2 monozygotic twins become concordant [11]. Furthermore, heterogeneity within Type 1 diabetes is suspected but has not yet been proven [12].

MODY is clinically separated from Type 2 diabetes by age-at-onset criteria and from Type 1 diabetes by its clear, dominant mode of inheritance. Whether MODY like Type 1 diabetes is linked to HLA has previously been unclear. Barbosa et al. [5] have claimed evidence for linkage between MODY and HLA (5, 13), although the lod scores were not significant ($z = 1.976$ for $\theta = 0.05$), whereas Nelson and Pyke [14] and Faber et al. [15] found no evidence for linkage, although no formal linkage analysis was performed in the two latter studies.

The clinical picture investigated in our study is very similar to those described by others. The lod scores found here excluded close linkage between MODY and HLA in this family. Whether this means that MODY is heterogeneous and consists of an HLA-linked and a non-HLA-linked form, or whether the positive lod scores found by Barbosa et al. [5] were a chance deviation, remains to be clarified.

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