DNA Insertion Sequences Near the Insulin Gene are not Associated with Maturity-Onset Diabetes of Young People

D. Owerbach¹, B. Thomsen⁴, K. Johansen², L. U. Lamm³ and J. Nerup²

¹Hagedorn Research Laboratory and ²Steno Memorial Hospital, Gentofte, ³Tissue Typing Laboratory, University Hospital, Aarhus, and

⁴Tissue Typing Laboratory, University Hospital, Copenhagen, Denmark

Summary. The association between DNA insertion sequences located near the insulin gene and the dominantly inherited maturity-onset diabetes of young people was studied in a large family. The distribution of the restriction fragments was compatible with Mendelian segregation. However, no linkage

was found between the DNA insertion sequences and this form of diabetes.

Key words: DNA insertion sequences, insulin gene, maturity-onset diabetes in young people.

A highly polymorphic region near the human insulin gene, which begins 363 base pairs before the start of transcription and extends upstream, is generated by variation in the number and arrangement of members of a family of tandemly repeated nucleotides whose structure is related to ACAGGGGTGTGGGG [1, 2, D. Owerbach, unpublished data].

Furthermore, the size variation is not random and the tandemly repeated sequences exist in two predominant types: less than 900 base pairs (bp) or greater than 1500 bp [1–7]. The larger class of repeated sequences, which we will refer to as large DNA insertions, have been suggested to play a role in glucose regulation [3] and to be associated with susceptibility to Type 2 (non-insulin-dependent) diabetes mellitus by Owerbach and Nerup [4, 5] and Rotwein et al. [6, 7] but not by Bell et al. [1] or Ullrich et al. [2]. However, pooling the available published data from all reports strongly indicates an association of the large DNA insertions and Type 2 diabetes ($p=0.00036$) [7].

Here we have investigated a large family having multiple members with maturity-onset type of diabetes in young people (MODY) with onset in adolescence, autosomal dominant inheritance, no or very little insulin requirement and very few microangiopathic complications.

Subjects and Methods

The kindred studied here consists of 56 members and was investigated in detail originally by Johansen and Gregersen [8]. The individual numbers in the pedigree (Fig. 1) are the same as the individual numbers in that report. Individuals 54 and 55 have been included since the original report. Blood samples were obtained from 17 diabetic members, one with impaired glucose tolerance, 23 unaffected members and 15 spouses. Some members of the family had renal glucosuria [36,

37, 41], but studies of renal thresholds were not performed in all subjects.

Restriction-Fragment Length Polymorphism

DNA isolated from white blood cells of participating family members was analysed by restriction-enzyme digestion and DNA blot analysis [4]. Briefly, DNA is isolated from nucleated blood cells and digested with the restriction endonuclease Bgl I. The fragments are separated by size on a 1% agarose gel and transferred to nitrocellulose filters by the method of Southern [9]. The DNA bound to the filters is then hybridized with a ³²P-labelled insulin-DNA probe. The probe was prepared from the genomic clone λ HI which was a gift from Drs. G.I. Bell and W.J. Rutter of California. The linkage between DNA fragments and MODY was evaluated by lod score analysis. Lod scores were calculated by application of the tables in Race and Sanger [10] to informative parts of the pedigree. Only completely typed individuals were included and therefore gene frequencies are irrelevant.

Results

Figure 2 shows an autoradiogram of the DNA sequences containing the insulin gene in DNA digested with Bgl I in 11 family members. In the family, four specific Bgl I restriction fragments of 2.8, 2.9 (L-alleles [4]), 4.6, and 4.8 kilobases (kb) (U-alleles [4]) are detected. The class of 4.6 and 4.8 kb restriction fragments contains the large tandemly repeated sequences (> 1500 bp). The presence of a single 2.8 or 2.9 kb sequence was determined by comparisons on the autoradiograms with individual patterns containing both sequences. For example, individual 21 has the 2.8 kb sequence while his father (number 6) has both a 2.8 kb and 2.9 kb sequence (Fig. 2). In addition, all analyses were repeated to ensure accurate determinations.

The distribution of restriction fragments within the family is compatible with Mendelian segregation of the alleles containing them. Six different genotypes were

Table 1. Insulin- and HLA-genotypes in diabetic and non-diabetic family members

No.	Diabetic patients		No.	Unaffected family members		No.	Spouses	
	Insulin genotype (kb)	HLA-A, C, B [9] paternal/maternal		Insulin genotype (kb)	HLA-A, C, B [9] paternal/maternal		Insulin genotype (kb)	HLA-A, C, B [9] paternal/maternal
2	2.8/2.9	2, w3, 40/28, w3, 40	7	2.8/2.9	2, w3, 40/1, w4, w35	A	2.9/2.9	1, -, 12/w32, w2, 40
6	2.8/2.9	2, w3, 40/28, w3, 40	9	2.8/2.9	2, w3, 40/28, w3, 40	B	4.6/4.6	1, -, 37/3, -, 14
8	2.8/2.9	2, w3, 40/28, w3, 40	11	2.8/2.9	2, -, w38/8, w3, 40	C	2.9/4.6	w24, w4, w35/28, w4, w35
12	2.8/2.9	2, -, w38/28, w3, 40	22	2.9/2.9	NT	D	2.9/2.9	28, -, 14/3, w4, w35
14	2.9/4.6	2, w3, 40/ , , ,	23	2.8/2.9	NT	E	2.9/4.6	w19, -, 7/2, -, w50
15	2.9/4.6	2, w3, 40/1, -, 8	24	2.9/2.9	NT	F	NT	28, w2, 27/1, -, 8
16	2.9/2.9	28, w3, 40/1, -, 12	25	2.9/2.9	NT	G	2.9/4.6	24, w3, 15/11, 4, 15
17	2.9/2.9	28, w3, 40/w32, w2, 40	26	NT	2, w3, 40/28, w4, w35	H	2.8/4.8	1, -, 8/28, w3, 40
19	2.8/2.9	1, -, 37/2, w3, 40	28	2.8/2.9	NT	I	2.9/4.6	NT
20	2.9/4.6	1, -, 37/2, w3, 40	29	2.8/4.6	NT	J	4.6/4.6	NT
21	2.8/4.6	1, -, 37, 28, w3, 40	32	2.9/4.6	w19, -, 7/2, w3, 40	K	2.9/2.9	NT
27	2.9/4.6	2, w3, 40/w24, w4, w35	33	2.9/4.6	28, w2, 27/2, w3, 40	L	2.8/2.9	NT
30	2.8/2.9	28, -, 14/28, w3, 40	34	2.9/2.9	1, -, 8/2, w3, 40	M	2.8/2.9	NT
31	2.9/2.9	w19, -, 7/2, w3, 40	35	2.9/4.6	1, -, 8/2, w3, 40	N	2.8/2.9	NT
36	4.6/4.6	1, -, 8/2, w3, 40	39	2.9/4.6	28, w3, 40/24, w3, 15	O	2.9/4.6	NT
37	2.9/4.6	28, w2, 27/1, -, 8	40	2.9/4.6	NT	P	2.9/2.9	NT
41 ^a	2.9/4.6	NT	44	2.9/2.9	NT			
43	2.9/4.8	1, -, 8/2, -, 7	45	2.9/2.9	NT			
			48	2.9/2.9	NT			
			49	2.8/2.9	NT			
			50	2.8/2.9	NT			
			53	2.9/4.6	NT			
			54	2.8/2.9	NT			
			55	2.9/4.6	NT			

^a Impaired glucose tolerance; NT=not tested

Fig. 1. Pedigree of the family. Diabetic = ■ male, ● female; impaired glucose tolerance = ◐ female; non-diabetic = □ male, ○ female; dead subjects = ☆. Figures denote family member numbers; letters indicate spouses

thus detected: 2.8/2.9, 2.9/2.9, 2.9/4.6, 2.8/4.6, 2.9/4.8, and 4.6/4.6 (Table 1).

By simple inspection, tight linkage between the 2.8, 2.9, 4.6, or 4.8 kb alleles and the MODY gene(s) is unlikely. All six genotypes are present in the diabetic members. Especially informative are diabetic individuals numbers 21 and 36 who contain 2.8/4.6 kb and 4.6/4.6 kb genotypes, respectively. They do not contain the 2.9 kb allele that is found in all other diabetic members of the family. Since individual number 2 has 2.8 and 2.9 kb alleles, the disease allele must be in coupling with the 2.9 kb allele in number 14 as well as in number 15. Accordingly, number 31 is a non-recombinant while both numbers 34 and 36 are recombinants; since the two matings 14 × E and 15 × F are intercusses, heterozygous offspring are uninformative. The lod scores obtained in this part of the pedigree appear in Table 2. Individual 6 is typed as a 2.8/2.9 heterozygote and since

B is a homozygous 4.6/4.6, the offsprings numbers 20 and 21 are also informative, showing one recombinant and one non-recombinant. This is scored by z_1 [10]. The pedigree gives only information on female recombination. The part of the family from which lod scores are derived includes only one healthy individual (number 34). In this individual, the disease could be firmly excluded by a normal haemoglobin A_{1c} (4.98%).

Table 1 shows the HLA-ACB types of the family members as reported previously [11]. HLA is linked to chromosome 6 [12] and the U- and L-alleles to chromosome 11 [13] and not surprisingly, the U- and L-alleles are not tightly linked to HLA (Table 1).

Discussion

This is the first investigation of DNA insertion sequences near the insulin gene in a family including mul-

Fig. 2. An autoradiogram of BglI DNA sequences containing the insulin gene in 11 family members. The relationship of the different individuals can be traced from Figure 1. Secondary restriction fragments resulting from incomplete digestion with BglI are detected and have been described previously [1–7]. These do not interfere with the correct interpretation of the primary restriction fragments indicated at 4.6, 2.9 and 2.8 kb

Table 2. Table of lod scores for selected recombination fractions between maturity onset of the young and DNA insertion sequences in part of a large kinship

Mating	Recombination fraction (θ)				
	0.05	0.1	0.2	0.3	0.4
14 × E 1nr: 0r ^a	0.279	0.255	0.204	0.146	0.079
15 × F 0nr: 2r	–2.000	–1.398	–0.796	–0.444	–0.194
6 × B z ₁ 1:1 ^b	–0.721	–0.444	–0.194	–0.76	–0.018
Total lod score	–2.442	–1.587	–0.786	–0.374	–0.133

^a nr = non-recombinants, r = recombinants; ^b z₁ score according to Race and Sanger [10]

multiple individuals with MODY. No association was found between diabetes and insertion sequences in this family.

It has been suggested that the class of large tandemly repeated sequences located near the insulin gene is associated with Type 2 diabetes [4–7]. We have previously examined the segregation of U- and L-alleles containing the insulin gene and associated repeated sequence in a large family having multiple insulin-dependent members and found the alleles to segregate in a Mendelian fashion [3]. In addition, we found the large insertion sequences to be associated with elevated haemoglobin A_{1c} levels in the non-diabetic members of the family [3]. In the present study a large MODY family was investigated. As in our first family study, the different sizes of restriction fragments containing the insulin gene segregate in a Mendelian fashion. In addition, the resolution of four different alleles allowed an analysis of their relationship to MODY. The presence of six different genotypes within the diabetic members alone demonstrates conclusively that the 2.8, 2.9, 4.6 or 4.8 kb alleles are not strongly associated with diabetes in this family.

Due to homozygosity, unfortunate genotype constellation and incomplete typing, the information regarding linkage between the insulin gene marker and the disease locus is rather meagre. The lod scores are,

however, negative for all recombination fractions considered, and if a score less than –2.0 is considered evidence against linkage [14], a recombination fraction of 0.08 or closer can be excluded.

The results are not inconsistent with previous findings of an association between the large-sized insertion sequences and Type 2 diabetes [4–7]. The diabetic patients in the previous study were older at diagnosis, generally obese, and had a high frequency of macroangiopathy [4]. Together, these observations suggest genetic heterogeneity within Type 2 diabetes.

Acknowledgement. We wish to thank Ms. L. Aagaard for excellent technical assistance.

References

1. Bell GI, Karam JH, Rutter WJ (1981) A polymorphic DNA region adjacent to the 5' end of the human insulin gene. *Proc Natl Acad Sci USA* 78: 5759–5763
2. Ullrich A, Dull TJ, Gray A, Philips JA, Peter S (1982) Variation in the sequence and modification state of the human insulin gene flanking regions. *Nucleic Acids Res* 10: 2225–2240
3. Owerbach D, Billesbølle P, Poulsen S, Nerup J (1982) DNA insertion sequences near the insulin gene affect glucose regulation. *Lancet* 1: 880–883
4. Owerbach D, Nerup J (1982) Restriction fragment length polymorphism of the insulin gene in diabetes mellitus. *Diabetes* 31: 275–277
5. Owerbach D, Johansen K, Billesbølle P, Poulsen S, Schroll M, Nerup J (1982). A possible association between DNA sequences flanking the insulin gene and atherosclerosis. *Lancet* 2: 1291–1293
6. Rotwein P, Chyn R, Chirgwin J, Cordell B, Goodman HM, Permutt MA (1981) Polymorphism in the 5'-flanking region of the human insulin gene and its possible relation to Type 2 diabetes. *Science* 213: 1117–1120
7. Rotwein PS, Chirgwin J, Province M, Knowler WC, Petitt DJ, Cordell B, Goodman H, Permutt AM (1983) Polymorphism in the 5' flanking region of the human insulin gene: a genetic marker for non-insulin-dependent diabetes. *New Engl J Med* 308: 65–71
8. Johansen K, Gregersen G (1977) A family with dominantly inherited mild juvenile diabetes. *Acta Med Scand* 201: 567–570
9. Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* 98: 503–517
10. Race RR, Sanger R (1968) Blood groups in man 5th edn. Blackwell, Oxford Edinburgh, p 502
11. Platz P, Jakobsen BK, Svejgaard A, Thomsen BS, Jensen KB, Henningsen K, Lamm LU (1982) No evidence for linkage between HLA and maturity onset type of diabetes in young people. *Diabetologia* 23: 16–18
12. Lamm LU, Friedrich U, Petersen GB, Jørgensen J, Nielsen J, Therkelsen AJ, Kissmeyer-Nielsen F (1974) Assignment of the major histocompatibility complex to chromosome No. 6 in a family with a pericentric inversion. *Human Hered* 24: 273–284
13. Owerbach D, Bell GI, Rutter WJ, Shows TB (1980) The Insulin gene is located on chromosome 11 in humans. *Nature* 286: 82–84
14. Morton N (1956) The detection and estimation of linkage between the genes for elliptocytosis and the Rh blood type. *Am J Hum Genet* 8: 80–96

Received: 5 October 1982

and in revised form: 25 March 1983

Dr. Jørn Nerup
Steno Memorial Hospital
DK-2820 Gentofte
Denmark