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Association of chosen microsatellite markers on chromosomes 10, 11p and 14q with IDDM susceptibility in the population of midwestern Poland

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Abstract. In search for new markers of insulin-dependent diabetes (IDDM) susceptibility we studied the CATT tetranucleotide repeat in intron 1 of tyrosine hydroxylase (TH) gene on chromosome 11p, the CA repeat at T-cell receptor α chain (TCRA) locus on chromosome 14q and two CA repeats at D10S211 and D10S213 loci in the chromosome 10 region containing glutamic acid decarboxylase (GAD2) gene. Alleles at these microsatellite loci were identified in a population of diabetic children and unrelated healthy controls originating from Wielkopolska, a midwestern region of Poland. We found significant association of certain alleles at TH, TCRA and D10S211 loci with diabetes in the population under study. On the contrary, none of the alleles at D10S213 locus was associated with the disease. Our findings indicate that typing of microsatellite markers may represent useful additional tool for identifying individuals at high risk of developing IDDM. Regarding loci on chromosome 10 our data and data published by other authors may suggest the extistence of two separate regions of association with IDDM susceptibility on this chromosome.

Key words: association, IDDM, microsatellite markers, susceptibility, PCR.

Introduction

Insulin-dependent diabetes mellitus (IDDM or type 1 diabetes) is a T-cell mediated autoimmune disease which results from a prolonged, selective and irreversible destruction of insulin-producing pancreatic β cells. The disease

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affects about 0.2-0.4% of Caucasian populations. Its onset is believed to be triggered by unknown environmental factors acting on a predisposing genetic background. The genetic component of type 1 diabetes is complex. Genes within the HLA region (locus IDDM1) on the short arm of chromosome 6 (6p21) are responsible for approximately 60 to 70% of genetic predisposition to IDDM. Another 30 to 40% of this predisposition is encoded by genes outside the HLA region (ROTTER, LANDAW 1984). Among the non-HLA genes the most important is locus IDDM2 in the insulin gene region on chromosome 11p15; several other susceptibility loci have been identified on chromosomes 15q (IDDM3), 11q (IDDM4), 6q (IDDM5) and 2q (IDDM7) (DAVIES et al. 1994, FIELD et al. 1994, HASHIMOTO et al. 1994, COPEMAN et al. 1995). Contribution of even more loci to susceptibility to IDDM cannot be excluded and among candidates are genes for immunoglobulin light and heavy chains (chromosomes 2p11 and 14q32.3, respectively), genes for T-cell receptor α and β subunits (chromosomes 14q11 and 7q35, respectively) (FIELD 1991, CAVAN et al. 1992) and chromosome 10 region containing glutamic acid decarboxylase (GAD2) gene (EDELHOFF et al. 1993, DAVIES et al. 1994). Identification of exact loci conferring genetic predisposition to type 1 diabetes is very important for subsequent prediction and prevention of the disease.

In search for new markers of IDDM susceptibility we focused our interest on microsatellites located closely to some candidate genes or genome regions known to predispose a child to developing IDDM. Microsatellites are abundant interspersed DNA elements in the genomes of many eukaryotes. They consist of a simple repetitive motif of 1-4 base pairs and are known to exhibit extensive length polymorphism resulting from a different number of repeats of a core sequence at a given locus. Therefore microsatellites are a rich new source of informative genetic markers (WEBER, MAY 1989, BECKMANN, WEBER 1992). Here we report results of our studies on the association of four microsatellite markers with IDDM susceptibility in the population of midwestern Poland, namely: the CATT tetranucleotide repeat in intron 1 of the tyrosine hydroxylase (TH) gene closely linked to the insulin gene on human chromosome 11p15.5, the CA dinucleotide repeat at the T-cell receptor α chain (TCRA) locus on chromosome 14q and two CA dinucleotide repeats at loci D10S211 and D10S213. We concentrated our research on the people of Wielkopolska, a midwestern Poland region inhabited by 14% of the whole country population. The incidence of IDDM in Wielkopolska region and more generally in Poland is 6-7/100,000 inhabitants.

Material and methods

IDDM patients and controls

101 subjects with IDDM and 97 unrelated healthy controls were selected for this study. Patients were recruited through Wielkopolska Childhood Diabetes Registry, in which approximately 14% of total number of Polish diabetic children are registered. All diabetics were from families with only one affected child. Diagnosis of IDDM was made at the local hospitals first and then confirmed at The Endocrinology Clinic of The Institute of Pediatrics in Poznań according to the generally accepted criteria, i.e. fasting plasma glucose exceeding 120 mg%, plasma glucose two hours after a meal exceeding 200 mg%, as well as classic symptoms of diabetes including polydipsia, polyuria and weight loss. Children were from 3 to 17 year-old at diagnosis (mean age of onset: 9.9 years). Informed consent for genetic studies was obtained from parents of diabetic children. Our controls were healthy unrelated adults with no family history of IDDM. HLA-DR and DQ alleles were identified in both groups; the results of HLA studies will be reported elsewhere (JUNGERMAN et al., in preparation).

Microsatellite marker typing

Allele sizes of microsatellite markers were determined by means of polymerase chain reaction (PCR) followed by acrylamide gel electrophoresis. We used oligonucleotide primers published by POLYMEROPOULOS et al. (1991) to amplify a 244 to 260 base pairs DNA fragment at the TH locus and primers published by CORNÉLIS et al. (1992) to amplify a 190 to 206 base pairs DNA fragment at the TCRA locus, both fragments containing the appropriate microsatellite repeat (Table 1). PCR amplifications were carried out in a 10-µl volume containing 40 ng of genomic DNA, 5 pmoles each oligodeoxynucleotide primer, 200 µM each dATP, dCTP, dGTP and dTTP, 5% DMSO, 10 mM Tris HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$ and 0.3 units of Taq polymerase (Boehringer Manheim). The samples were overlaid with mineral oil and were processed through 35 temperature cycles consisting of denaturation for 30 sec. at 94°C, annealing for 45 s at 55°C and primer extension for 45 sec. at 72°C. First denaturation and last elongation steps were lengthened to 3 and 4 min, respectively. To resolve the alleles aliquots of amplified samples were electrophoresed on standard denaturing polyacrylamide DNA sequencing gels (6%

polyacrylamide, 7M urea). DNA was visualized by silver staining (BASSAM et al. 1991). DNA size standards were dideoxy sequencing ladders produced using pGEM®-3Zf(+) vector (Promega) as a template.

To amplify the CA dinucleotide repeats at D10S211 and D10S213 loci we used oligonucleotide primers published in The Généthon Microsatellite Map Catalogue (1992) (Table 1). Polymerase chain reactions were carried out in a 25-µl volume containing 100 ng of genomic DNA, 15 pmoles each oligodeoxynucleotide primer, 200 µM each dATP, dGTP and dTTP, 2.5 µM dCTP, 1 µCi $\alpha[^{32}P]$dCTP at 3,000 Ci/mmol, 10% DMSO, 10 mM Tris HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$ and 0.3 units of Taq polymerase (Boehringer Manheim). The samples were overlaid with mineral oil and were processed through 30 temperature cycles (1 min at 94°C, 1 min at 55°C and 1 min at 72°C). First denaturation and last elongation steps were lengthened to 4 min.

Table 1. Nucleotide sequence of primers used for enzymatic amplification of microsatellite markers

Locus	Primer sequences (5' → 3')
TH	CAGCTGCCCTAGTCAGCAC GCTTCCGAGTGCAGGTCACA
TCRA	ATCTCCCTGCCCTCATATAG CATGCCACCCATAACCAACC
D10S211	CTCCTGGTCTCATGCG CAGGCTCCTACTACCGTC
D10S213	AGCATGTCTCTGACGGCTC CAACATCAATAGTGGGACTTTGC

Aliquots of amplified samples were electrophoresed on standard denaturing polyacrylamide DNA sequencing gels as above. Gels were dried at 80°C and autoradiographed for 24 to 72 hrs at a room temperature without intensifying screens. DNA size standards were PCR products obtained using genomic DNA of the mother of the CEPH family 1347 (individual 134702). The allele sizes for this individual are published in The Généthon Microsatellite Map Catalogue (1992) and therefore 134702 can be used as a reference for allele size determination.

Statistical analysis

Allele frequencies were compared between patients and unrelated healthy controls. We compared the following number of individuals (diabetics vs.

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Statistical analysis

Allele frequencies were compared between patients and unrelated healthy controls. We compared the following number of individuals (diabetics vs.

controls): at the TH locus – 98 vs. 97, at the TCRA locus – 101 vs. 97, at the D10S211 locus – 85 vs. 82 and at the D10S213 locus – 94 vs. 97, respectively. PCR products which despite repeating the experiments had not been synthesized efficiently or had not been resolved on gels clearly enough to determine band sizes, were not incorporated in data sets used for statistical analysis. Comparisons of alleles were made by χ^2 analysis with Yates continuity correction. Fisher's exact test was used when sample number was < 3, P values were corrected for a number of comparisons and considered significant when < 0.05. The relative risk was estimated according to Woolf's formula, with Haldane's modification in sets containing 0 (WOOLF 1955, HALDANE 1956, TIWARI, TERASAKI 1985).

Results

At the TH locus five alleles can be identified depending on the number of CATT repetitive sequence in intron 1 of the tyrosine hydroxylase gene (Table 2). In our healthy controls the most frequent allele was 260 bp (38.6%)

Table 2. Allele frequencies at the CATT tetranucleotide repeat locus in the tyrosine hydroxylase (TH) gene

Allele size (base pairs)	IDDM n (%)	Controls n (%)
244	53 (27.0)	50 (25.8)
248	31 (15.8)	31 (16.0)
252	23 (11.7)	11 (5.7)
256	49 (25.0)[a]	27 (13.9)
260	40 (20.4)[b]	75 (38.6)
Total	196	194

a: $\chi^2 = 7.63$; $p_c = 0.03$; RR = 2.06; 95% C.I. = [1.19-3.61]

b: $\chi^2 = 15.62$; $p_c = 0.0004$; RR = 0.41; 95% C.I. = [0.25-0.65]

and the least frequent – 252 bp (5.7%). Two of the TH alleles were significantly associated with diabetes in the population under study: 256 base pairs allele was increased in diabetics and 260 bp allele was decreased in diabetics (RR = 2.06, $p_c = 0.03$; RR = 0.41, $p_c = 0.0004$, respectively; Table 2).

Table 3. Allele frequencies at the CA dinucleotide repeat at TCRA locus

Allele size (base pairs)	IDDM n (%)	Controls n (%)
190	1 (0.5)	0
192	1 (0.5)	4 (2.1)
194	54 (26.7)	51 (26.3)
196	13 (6.4)[a]	2 (1.0)
198	37 (18.3)	38 (19.6)
200	68 (33.7)	82 (42.3)
202	22 (10.9)	14 (7.2)
204	5 (2.5)	3 (1.5)
206	1 (0.5)	0
Total	202	194

a : $\chi^2 = 7.93$; $p_c = 0.043$; RR = 6.60; 95% C.I. = (1.46-60.83)

Table 4. Allele frequencies at the D10S211 dinucleotide repeat locus

Allele size (base pairs)	IDDM n (%)	Controls n (%)
195	38 (22.3)	29 (17.7)
197	17 (10.0)	22 (13.4)
199	19 (11.2)	31 (18.9)
201	41 (24.1)[a]	20 (12.2)
203	9 (5.3)[b]	29 (17.7)
205	9 (5.3)	9 (5.5)
207	21 (12.4)	14 (8.5)
209	14 (8.2)	8 (4.9)
211	2 (1.2)	2 (1.2)
Total	170	164

a: $\chi^2 = 7.95$; $p_c = 0.04$; RR = 2.29; 95% C.I. = (1.23-4.34)

b: $\chi^2 = 12.71$; $p_c = 0.003$; RR = 0.26; 95% C.I. = (0.11-0.59)

Table 5. Allele frequencies at the D10S213 dinucleotide repeat locus

Allele size (base pairs)	IDDM n (%)	Controls n (%)
167	4 (2.1)	2 (1.0)
171	0	1 (0.5)
173	7 (3.7)	3 (1.5)
175	1 (0.5)	9 (4.6)
177	17 (9.1)	15 (7.7)
179	8 (4.3)	14 (7.2)
181	34 (18.1)	19 (10.0)
183	13 (6.9)	15 (7.7)
185	23 (12.2)	25 (12.9)
187	55 (29.3)	55 (28.4)
189	19 (10.1)	28 (14.4)
191	7 (3.7)	7 (3.6)
195	0	1 (0.5)
Total	188	194

At the TCRA locus we observed nine alleles depending on the number of CA dinucleotide repeats (Table 3). In healthy controls the most frequent allele was 200 bp (42.3%), and the least frequent – 196 bp (1.0%). The 196 bp allele was significantly increased in diabetics in our population (RR = 6.60, p_c = 0.043; Table 3).

The CA microsatellite repeat of the D10S211 locus was detected to have nine alleles (Table 4). In the control group the most frequent allele was 199 bp (18.9%) and the least frequent – 211 bp (1.2%). Two of the alleles were significantly associated with the disease in our population: 201 bp allele was increased in diabetics and 203 bp allele was decreased in diabetics (RR = 2.29, p_c = 0.04; RR = 0.26, p_c = 0.003, respectively; Table 4).

The CA microsatellite repeat of the D10S213 locus was detected to have thirteen alleles (Table 5). We found none of them being significantly associated with diabetes in the population under study. In our controls the most frequent

Table 6. Microsatellite markers on chromosome 10 shown in order according to DAVIES et al. (1994) and The Généthon Microsatellite Map Catalogue and their relation to IDDM susceptibility

Microsatellite marker	Relation to IDDM susceptibility
D10S191	not linked*
D10S211	**associated**
D10S197	linked*
D10S213	**not associated**
D10S193	linked*
D10S220	linked*
D10S539	not linked*

Markers shown in bold letters are described in this paper. Data indicated by asterisk are taken from DAVIES et al. (1994).

allele was 187 bp (28.4%), whereas the least frequent alleles were 171 and 195 bp (both at 0.5%).

Comparison of allele frequencies between males and females within diabetic group and between males and females within control group showed no differences of these frequencies between sexes in both groups at all loci under study (data not shown).

Discussion

Up till now no broad research of genetic markers of inherited predisposition to insulin-dependent diabetes mellitus in Polish population has been conducted. In order to fill this gap we collected and began to study more than 100 DNA samples from diabetic children and 97 unrelated healthy controls. All diabetics as well as healthy subjects originate from Wielkopolska which is a region of midwestern Poland with demographic features making it representative for the whole country. Both groups did not match each other with respect to age. We selected adults for the control group because it was known they had not developed diabetes at a younger age, whereas in the case of children the chance that some of them would develop diabetes later in their lives would be higher. We examined the microsatellite tetranucleotide CATT repeat within intron 1 of tyrosine hydroxylase gene on the short arm of chromosome 11 (11p15.5), for which we were able to identify five alleles. A significantly decreased frequency of 260 bp allele and increased frequency of 256 bp allele was observed among IDDM patients compared to controls in our study, which is in agreement for the 260 bp allele with LUCASSEN et al. (1993) (allele sizes given in the original paper by Lucassen are different because of a different set of oligonucleotide primers used for PCR; our 260 bp allele corresponds to their 122 bp allele). We observed no difference in the frequencies of the 244 bp allele in diabetics and healthy controls, which had been previously reported to be associated with IDDM by HEARNE et al. (1992). This may be due to genetic differences of

geographically distant populations. At this locus, as well as at other loci discussed below, allele frequencies were not influenced by sex of probands, since no differences of these frequencies were found between sexes within diabetic and control groups.

For the CA dinucleotide repeat at the T-cell receptor α chain (TCRA) locus on chromosome 14q we identified seven alleles in healthy controls and nine alleles in diabetic children. The frequency of two alleles detected exclusively in diabetics (190 and 206 bp) was very low (0.5%). We found a significant increase in the frequency of 196 bp allele in diabetics (6.4% in IDDM patients vs. 1.0% in controls), however, because of a very broad range of C.I. value (C.I. = [1.46-60.83]) further studies of more numerous populations of diabetic children as well as healthy controls are necessary to confirm this finding. The association of the TCRA locus with susceptibility to insulin-dependent diabetes mellitus has not been reported so far by other investigators.

We studied CA dinucleotide repeats at two loci on chromosome 10, namely D10S211 and D10S213. These loci were chosen for studies because of their location in the region of chromosome 10 (between 10p13 and 10q11.2) which contains GAD2 gene coding for glutamic acid decarboxylase. The GAD2 gene, which is expressed in human pancreatic islets and brain, has been mapped to chromosome 10p11.3-p13 (KARLSEN et al. 1991, EDELHOFF et al. 1993). Glutamic acid decarboxylase (GAD) catalyzes the synthesis of γ-aminobutyric acid (GABA), which is known as a major inhibitory neurotransmitter in the central nervous system (CNS), but is also present outside CNS. GAD autoantibodies have been detected in patients years before the clinical onset of the IDDM (BAEKKESKOV et al. 1987, ATKINSON et al. 1990) suggesting that GAD is the autoantigen involved in the development of the disease (BAEKKESKOV et al. 1982, 1990). Immune response to GAD has been also shown to correlate with insulitis in non-obese diabetic mice, a murine model for spontaneous IDDM (TISCH et al. 1993). Linkage of several microsatellite loci on chromosome 10 to IDDM has been already shown by some authors (DAVIES et al. 1994).

At D10S211 we identified nine alleles, two of them being significantly associated with IDDM. We did not observe any significant differences in the frequencies of alleles at D10S213 between IDDM patients and healthy controls. Two of the alleles observed at a very low frequency in controls were absent in diabetic children (171 bp and 195 bp). They could probably be detected in a larger group of diabetics. Some positive evidence for the linkage of this region of chromosome 10 with insulin-dependent diabetes mellitus has been already described by others (DAVIES et al. 1994), who studied some multiplex IDDM families (more than one affected child). D10S213 microsatellite which we describe here as not associated with IDDM susceptibility lies between

D10S197 and D10S193 which, according to these authors, are linked to the disease. Our D10S211 marker associated with IDDM susceptibility is adjacent to D10S197, but lies on the other side of D10S197, than D10S213 (Table 6). All these data may suggest the existence of two regions associated with IDDM susceptibility on chromosome 10: one region marked by D10S211 and D10S197 and the other marked by D10S193 and D10S220, separated by non-associated marker D10S213. Studies of D10S213 marker in different populations of diabetic children and healthy subjects might be useful for confirmation of our finding.

Conclusions

We conclude that associations of CATT microsatellite in the tyrosine hydroxylase gene as well as CA microsatellites at the T-cell receptor α chain (TCRA) and D10S211 loci with diabetes in Polish population result from the involvement of particular regions of chromosomes 11p, 14q and 10, respectively, in rendering a child susceptible to IDDM. For the TH marker this may be due to a strong linkage disequilibrium of tyrosine hydroxylase gene with insulin gene. For D10S211 the hypothetical linkage disequilibrium of this marker and GAD2 gene might be the explanation, however the identification of D10S213 as a non-associated marker suggests the presence of two separate regions of association with IDDM susceptibility on chromosome 10. We feel that data obtained for the TCRA marker require confirmation on larger groups of diabetics and controls, but findings concerning TH and D10S211 markers are clear and typing of these two markers may represent a useful additional tool for identifying individuals at high risk of developing IDDM.

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REFERENCES

ATKINSON M.A., MACLAREN N.K., SCHARP D.W., LACY P.E., RILEY W.J. (1990). 64,000 M_r autoantibodies as predictors of insulin-dependent diabetes. Lancet 335: 1357-1360.

BAEKKESKOV S., AANSTOOT H.-J., CHRISTGAU S., REETZ A., SOLIMENA M., CASCALHO M., FOLLI F., RICHTER-OLESON H., De CAMILLI P. (1990). Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. Nature 347: 151-156.

BAEKKESKOV S., LANDIN-OLSSON M., KRISTENSEN J.K., SRIKANTA S., BRUINING G.J., MANDRUP-POULSEN T., De BEAUFORT C., SOELDNER J.S., EISENBARTH G., LINDGREN F., SUNDKVIST G., LERNMARK Å. (1987). Antibodies to a Mr 64,000 human islet cell antigen precede the clinical onset of insulin-dependent diabetes. J. Clin. Invest. 79: 926-934.

BAEKKESKOV S., NIELSEN J.H., MARNER B., BILDE T., LUDVIGSSON J., LERNMARK Å. (1982). Autoantibodies in newly diagnosed diabetic children immunoprecipitate human pancreatic islet cell proteins. Nature 298: 167-169.

BASSAM B.J., CAETANO-ANOLLES G., GRESSHOFF P.M. (1991). Fast and sensitive silver staining of DNA in polyacrylamide gels. Analytical Biochemistry 196: 80-83.

BECKMANN J.S., WEBER J.L. (1992). Survey of human and rat microsatellites. Genomics 12: 627-631.

CAVAN D., BAIN S., BARNETT A. (1992). The genetics of type I (insulin dependent) diabetes mellitus. J. Med. Genet. 29: 441-446.

COPEMAN J.B., CUCCA F., HEARNE C.M., CORNALL R.J., REED P.W., RØNNINGEN K.S., UNDLIEN D.E., NISTICO L., BUZZETTI R., TOSI R. (1995). Linkage disequilibrium mapping of a type 1 diabetes susceptibility gene (IDDM7) to chromosome 2q31-q33. Nature Genet. 9: 80-85.

CORNÉLIS F., HASHIMOTO L., LOVERIDGE J., MACCARTHY A., BUCKLE V., JULIER C., BELL J. (1992). Identification of a CA repeat at the TCRA locus using yeast artificial chromosomes: a general method for generating highly polymorphic markers at chosen loci. Genomics 13: 820-825.

DAVIES J.L., KAWAGUCHI Y., BENNETT S.T., COPEMAN J.B., CORDELL H.J., PRITCHARD L.E., REED P.W., GOUGH S.C.L., JENKINS S.C., PALMER S.M., BALFOUR K.M., ROWE B.R., FARRALL M., BARNETT A.H., BAIN S.C., TODD J.A. (1994). A genome-wide search for human type 1 diabetes susceptibility genes. Nature 371: 130-136.

EDELHOFF S., GRUBIN C.E., KARLSEN A.E., ADLER D.A., FOSTER D., DISTECHE C.M., LERNMARK Å. (1993). Mapping of glutamic acid decarboxylase (GAD) genes. Genomics 17: 93-97.

FIELD L.L. (1991). Non-HLA region genes in insulin dependent diabetes mellitus. Bailliere's Clin. Endocrinol. Metab. 5: 413-437.

FIELD L.L., TOBIAS R., MAGNUS T. (1994). A locus on chromosome 15q26 (IDDM3) produces susceptibility to insulin-dependent diabetes mellitus. Nature Genet. 8: 189-194.

HALDANE S. (1956). The estimation and significance logarithm of ratio of frequencies. Ann. Hum. Genet. 20: 309-311.

HASHIMOTO L., HABITA C., BERESSI J.P., DELEPINE M., BESSE C., CAMBON-THOMSEN A., DESCHAMPS I., ROTTER J.I., DJOULAH S., JAMES M.R., FROGUEL P., WEISSENBACH J., LATHROP G.M., JULIER C. (1994). Genetic mapping of a susceptibility locus for insulin-dependent diabetes mellitus on chromosome 11q. Nature 371: 161-164.

HEARNE C.M., GHOSH S., TODD J.A. (1992). Microsatellites for linkage analysis of genetic traits. Trends Genet. 8: 288-294.

JUNGERMAN M., SANCHEZ-MAZAS A., FICHNA P., HORS J., CHARRON D., DJOULAH S. HLA class II DRB1, DQA1 and DQB1 SSP polymorphisms in the Polish population from Wielkopolska (in preparation).

JUNGERMAN M., FICHNA P., HORS J., CHARRON D., DJOULAH S. Analysis of HLA-DRB1, DQA1 and DQB1 genes in Polish insulin-dependent diabetes patients (in preparation).

KARLSEN A.E., HAGOPIAN W.A., GRUBIN C.E., DUBE S., DISTECHE C.M., ADLER D.A., BÄRMEIER H., MATHEWES S., GRANT F.J., FOSTER D., LERNMARK Å. (1991). Cloning and primary structure of a human islet isoform of glutamic acid decarboxylase from chromosome 10. Proc. Natl. Acad. Sci. USA 88: 8337-8341.

LUCASSEN A.M., JULIER C., BERESSI J.-P., BOITARD C., FROGUEL P., LATHROP M., BELL J.I. (1993). Susceptibility to insulin dependent diabetes mellitus maps to a 4.1 kb segment of DNA spanning the insulin gene and associated VNTR. Nature Genet. 4: 305-310.

POLYMEROPOULOS M.H., XIAO H., RATH D.S., MERRIL C.R. (1991). Tetranucleotide repeat polymorphism at the human tyrosine hydroxylase gene. Nucleic Acids Res. 19: 3753.

ROTTER J.I., LANDAW E.M. (1984). Measuring the genetic contribution of a single locus to a multilocus disease. Clin. Genet. 26: 529-542.

TISCH R., YANG X.-D., SINGER S.M., LIBLAU R.S., FUGGER L., MCDEVITT H.O. (1993). Immune response to glutamic acid decarboxylase correlates with insulitis in non-obese diabetic mouse. Nature 366: 72-75.

TIWARI J.L., TERASAKI P.I. (1985). The data and statistical analysis. In: HLA and disease associations (Tiwari J.L., Terasaki P.I., eds.). Springer, New York, 18-37.

WEBER J.L., MAY P.E. (1989). Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am. J. Hum. Genet. 44: 388-396.

WOOLF B. (1955). On estimating the relation between blood group and disease. Ann. Hum. Genet. 19: 251-253.