Concordance for Type 1 Diabetes in Identical Twins Is Affected by Insulin Genotype

KARL A. METCALFE, MRCP[1]
GRAHAM A. HITMAN, MD[1]
RACHEL E. ROWE, MD[1]
MOHAMMED HAWA, BSC[1]
XIAOJIAN HUANG, PHD[2]
TIMOTHY STEWART, PHD[2]
R. DAVID G. LESLIE, MD[1]

OBJECTIVE — Monozygotic twins are usually discordant (only one twin affected) for type 1 diabetes. Discordance for disease between such twins implies a role for nongenetically determined factors but could also be influenced by a decreased load of diabetes susceptibility genes. The aim of this study was to determine whether two susceptibility genes were less prevalent in discordant twins compared with concordant twins.

RESEARCH DESIGN AND METHODS — We studied 77 monozygotic twin pairs (INS), 40 concordant and 37 discordant, for type 1 diabetes at polymorphism of the insulin gene region on chromosome 11p and HLA-DQBI.

RESULTS — The disease-associated INS genotype (*Hph I*) was identified in 87.5% of the concordant twins but only in 59.5% ($P = 0.005$) of the discordant twins. Neither DQB1*0201 nor DQB1*0302 was seen in 2 of 40 (5%) concordant twins compared with 8 of 37 (22%) discordant twins ($P = 0.04$). No statistical differences were seen between concordant and discordant twins at individual alleles of DQB1. Combining insulin and DQ data, 5% of concordant twins compared with 32.4% of discordant twins had neither DQB1*0201/DQB1*0302 nor the high-risk *Hph I* INS "++" genotype ($P = 0.002$).

CONCLUSIONS — We conclude that the possession of the high-risk *Hph I* insulin genotype increases the likelihood of identical twins being concordant for type 1 diabetes and that the "load" of both major histocompatibility complex (MHC) and non-MHC susceptibility genes has an impact on the disease penetrance of type 1 diabetes.

Diabetes Care 24:838–842, 2001

Concordance rates for type 1 diabetes are higher in monozygotic than dizygotic twins, which is consistent with a role for genetic factors determining the disease (1). Nevertheless, ~50% of monozygotic twins are discordant for type 1 diabetes, providing strong evidence that nongenetically determined factors also influence susceptibility to disease.

At least 30% of the genetic susceptibility to type 1 diabetes can be explained by an association with the major histocompatibility complex (MHC) (2). Although a large number of non-MHC chromosomal regions have been implicated in disease risk, only a few with known function have been identified, including the insulin gene hypervariable region (2–5), the interleukin gene cluster (6,7), CTLA4 (8–10), and the vitamin D receptor (11,12). However, the only consistent association between type 1 diabetes and a non-MHC region is with the insulin gene.

The study of monozygotic twins concordant or discordant for type 1 diabetes is a potentially powerful method to determine whether disease penetrance is a random effect or influenced by genetic factors. Monozygotic twins tend to develop type 1 diabetes within 6 years of each other (13), so that twin pairs discordant for diabetes are likely to remain discordant. We identified such discordant twin pairs in a previous HLA phenotype study and showed that they were less likely than concordant pairs to have both HLA-DR3 and HLA-DR4 antigens (14). Subsequent studies indicated that pairs remaining discordant are more accurately defined as nondiabetic twins more than 11 years from the diagnosis of the index twin, with normal glucose tolerance and without antibody markers (15–17). Because there are no previous genotype studies of HLA-DQB1 or non–MHC-encoded susceptibility genes to type 1 diabetes in identical twins, we have now tested discordant as well as concordant pairs using this definition of discordance. Our hypothesis was that discordance, as compared with concordance, for type 1 diabetes in monozygotic twins is caused by a decreased load of both MHC and non-MHC susceptibility genes.

RESEARCH DESIGN AND METHODS

— We have studied and compared 40 concordant and 37 discordant monozygotic twins for type 1 diabetes at both HLA-DQBI and the insulin gene (INS) loci. Twins studied were selected blind to those included in our previous study 15 years ago of HLA-DR phenotype (14). A total of 54% of twin pairs were common to both studies.

Twins were ascertained from 1967 to

From the [1]Department of Diabetes and Metabolic Medicine, St. Bartholomew's and the Royal London School of Medicine and Dentistry, Queen Mary and Westfield College, University of London, London, U.K.; and [2]Genentech, South San Francisco, California.

Address correspondence and reprint requests to Graham A. Hitman, MD, Department of Diabetes and Metabolic Medicine, Medical Unit, The Royal London Hospital, Whitechapel, London E1 1BB, U.K. E-mail: G.A.Hitman@mds.qmw.ac.uk.

Received for publication 8 May 2000 and accepted in revised form 21 December 2000.

X.H. and T.S. are employed by and hold stock in Genentech. Genentech is engaged in the development of pharmaceuticals for the treatment of diabetes and its complications.

Abbreviations: ICA, islet cell antibody; MHC, major histocompatibility complex.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

1997 by referral to the British Diabetic Twin Study; monozygosity was established in each twin pair as described previously (18,19). DNA was not available from all twins. All twins were invited to attend the Diabetes Department for blood sampling, but in the study period, only a fraction were able to do so. We therefore selected twin pairs who had attended the Diabetes Department consecutively from 1995 to 1997, who were either concordant or discordant for type 1 diabetes. The diabetic index twin from both concordant and discordant pairs had a comparable age at diagnosis, and nondiabetic twins from discordant pairs had a low risk of developing diabetes. Discordant pairs likely to remain discordant were identified by being >11 years from the diagnosis of the index twin and, at 11 years by having normal glucose tolerance and the absence of islet cell antibody (ICA), GAD antibody, or IA-2ic antibody.

Of 247 concordant twin pairs, we selected a consecutive series of 40 pairs attending the Diabetes Center (mean age of diagnosis of index twin 16 ± 9 years, range 1–39; 16 male pairs; median time to concordance 3.95 years, range 0.4–25). Of 109 discordant twin pairs, we identified a consecutive series of 37 pairs (mean age of diagnosis of index twin 19 ± 11 years, range 2–59; 20 male pairs) in which the index twin had a comparable age at diagnosis to the index twin of the concordant pairs. Nondiabetic twins underwent oral glucose tolerance testing (glucose was given as 75 g or 1.75 g/kg, whichever was less) to confirm that they had neither diabetes nor impaired glucose tolerance both initially and at intervals thereafter; diagnosis of type 1 diabetes was made according to standard guidelines (20). Sera were stored at –20°C and analyzed for each of three antibody assays. Analysis was performed on batched samples by observers blinded to the clinical status of the subjects.

Autoantibody assays

IA-2ic and GAD_{65} were measured by radioimmunoprecipitation assays as described previously (Promega, Madison, WI) (17). All samples were tested in duplicate, including positive and negative control standard sera. Each assay for IA-2ic and GAD_{65} antibodies included serially diluted sera from a patient with stiff-man syndrome and a prediabetic individual (a twin who subsequently developed type 1 diabetes but was not part of this study) to further evaluate the cutoff level for positivity for GAD_{65} and IA-2ic. Levels >3 SD higher than that of a control population were considered positive, as described previously (17). In the latest IA-2ic (unpublished) and GAD_{65} antibody proficiency workshops, our assays had a sensitivity, specificity, validity, and consistency of 100% in each (21).

Undiluted sera were screened for ICA. The presence of ICA was established by testing serum with indirect immunofluorescence on a fresh group O cryofixed human pancreas (16). Positive tests were defined as ≥4 Juvenile Diabetes Foundation units. The ICA assay assessed in the ENDIT workshop (unpublished) had a sensitivity and specificity of 100%.

HLA-DQBI typing

HLA-DQBI was studied by a polymerase chain reaction restriction fragment–length polymorphism method combined with group-specific primers adapted from Nomura et al. (22). DNA was extracted from blood (23), and a 241-bp region from the polymorphic second exon of the DQB1 gene was amplified in two separate PCRs, specific for DQwI and DQw2,3,4 alleles. Seven DQwI and six DQw2,3,4 alleles can be identified after restriction against panels of seven and five restriction enzymes, respectively (22,24). The digested and undigested products were visualized by ethidium bromide staining after electrophoresis in a 4.2% agarose gel.

INS

INS was studied at the 1,127 *Pst I* polymorphic restriction site in the 3′ untranslated region of the insulin gene and at the −23 *Hph I* polymorphic restriction site (3). The digested and undigested products were visualized by ethidium bromide staining after electrophoresis in a 4.2% agarose gel. By convention, the disease associated allele at both sites is denoted as "+."

Table 1—*HLA-DQB1 allele positivity in monozygotic twins with type 1 diabetes*

Allele	% Concordant (n = 40)	% Discordant (n = 37)	P value
0201	82.5 (n = 33)	65 (n = 24)	0.08
0301	2.5 (n = 1)	5.4 (n = 2)	NS
0302	60 (n = 24)	40.5 (n = 15)	0.09
0402	—	5.4 (n = 2)	NS
0501	—	2.7 (n = 1)	NS
0502	13 (n = 5)	5.4 (n = 2)	NS
0601	—	2.7 (n = 1)	NS
0602/3	2.5 (n = 1)	5.4 (n = 2)	NS
0604	—	5.4 (n = 2)	NS
0201/0302	47.5 (n = 19)	27 (n = 10)	0.06
Negative for DQB1*201 and/or *0302	5 (n = 2)	22 (n = 8)	0.04

NS, not significant.

Table 2—−23 Hph I *insulin genotypes in monozygotic twins with type 1 diabetes*

	INS Genotypes	
	+/+	+/− (plus −/−)
Concordant twins (n = 40)	0.875 (n = 35)	0.125 (n = 5)
Discordant twins (n = 37)	0.595 (n = 22)	0.405 (n = 15)
British caucasoid, ICA-negative controls (n = 63)*	0.54 (n = 34)	0.46 (n = 29)
Type 1 diabetes (n = 116)*	0.72 (n = 83)	0.28 (n = 33)

Concordant twins versus discordant twins (P = 0.005); concordant twins versus controls (P = 0.0001); concordant twins versus type 1 diabetes (P = 0.054); discordant twins versus controls (P = 0.68); discordant twins versus type 1 diabetes (P = 0.22); type 1 diabetes versus controls (P = 0.022). *Results taken from ref. 31. Patients with type 1 diabetes were serially selected from the Barts Oxford study, which is a population-based study of type 1 diabetes in the Oxfordshire region. The controls were all ICA-negative and were aged-matched to the subjects with type 1 diabetes. The disease-associated allele is designated (+).

Table 3—Hph I *high-risk insulin genotype and HLA DQB1*0201/*0302 combinations in monozygotic twins with type 1 diabetes*

Twins	INS negative DQ negative*	INS positive DQ positive	INS negative DQ positive	INS positive DQ negative
Concordant (n = 40)	0.05 (n = 2)	0.40 (n = 16)	0.075 (n = 3)	0.475 (n = 19)
Discordant (n = 37)	0.324 (n = 12)	0.189 (n = 7)	0.081 (n = 3)	0.405 (n = 15)

INS, possession of *Hph I* high-risk genotype (+/+); DQ, possession of HLA DQB1*0201 and/or *0302. Overall Pearson χ^2, P = 0.012; likelihood exact χ^2, P = 0.011. *2 × 2 Pearson χ^2, P = 0.002.

Statistics

All statistical analyses were performed using SPSS for Windows software (version 9; Chicago, IL). Differences in frequencies of genotypes and alleles were calculated by χ^2 analysis; all P values quoted are two-sided. Power analysis based on a 31% difference of DR3/DR4 heterozygotes between concordant and discordant twins, as observed in our previous study (14), indicated that 37 twin pairs in each group would be sufficient to show a difference at the 5% level with 80% power. The study was approved by the ethical committee of the Barts and the London National Health Service Trust. Written and informed consent was received from the twins or their parents, as appropriate.

RESULTS

HLA DQBI gene

Results are presented in Table 1. No differences were found between concordant and discordant twins for positivity of DQB1*0302, DQB1*0201, the combination of DQB1*0302/0201, and DQBI*0602/3. In contrast, 2 of 40 (5%) concordant twins compared with 8 of 37 (22%; P = 0.04) discordant possessed neither DQB1*0201 nor DQB1*0302.

INS

Results are presented in Table 2. The type 1 diabetes–associated +/+ genotype was increased for the 5′ *Hph I* (P = 0.005; Table 2) polymorphism. Similarly, the 3′ *Pst I* INS polymorphism was increased in frequency in concordant twins (frequency of +/+ genotype was 87.5% in concordant twins and 65% in discordant twins; P = 0.035).

HLA DQB1 and INS combined

Results are presented in Table 3. A total of 2 of 40 (5%) concordant twins compared with 12 of 37 (32.4%) discordant twins possessed neither DQB1*0201/DQB1*0302 nor the high-risk *Hph I* INS +/+ genotype (P = 0.002).

CONCLUSIONS— Our hypothesis was that discordance, compared with concordance, for type 1 diabetes in monozygotic twins is caused by a decreased load of both MHC and non-MHC susceptibility genes. From our observations, we conclude that the "load" of both MHC and non-MHC susceptibility genes does have an impact on disease penetrance of type 1 diabetes. Thus, the 5′ *Hph I* locus of the insulin gene was more prevalent in concordant than discordant twin pairs. Furthermore, there was an increased frequency of no high-risk HLA-DQB1 alleles in discordant compared with concordant twins. HLA-DQ protective alleles were as prevalent in concordant pairs as in discordant pairs. Lastly, only 5% concordant vs. 32.4% discordant twin pairs with type 1 diabetes possessed neither the HLA-DQB1*0302/0201 combination nor the INS *Hph I* high-risk genotype.

Differences between monozygous twins cannot be determined by germ-line genes. However, there are a number of ways genes could contribute to such disease discordance, including an increase in either protection or susceptibility to the disease. In the absence of a comprehensive understanding of the genes that contribute to genetic susceptibility to diabetes, the analysis of monozygotic twins discordant and concordant for diabetes provides a powerful method to assess the impact of a particular gene on disease expression.

Using DQB1 typing, we have confirmed the importance of the total load of MHC alleles as a determinant of concordance of disease, although we have been unable to confirm our previous results by studying individual DQB1 alleles compared with the previous DR serological typing (14). In that previous study, we found an increased frequency of the heterozygous phenotype HLA-DR3 and HLA-DR4 (linked to HLA-DQB1*0201 and DQB*0302, respectively) in concordant compared with discordant monozygotic twins. Several explanations are possible for the discrepant findings. First, there is a need to study a larger sample population to confirm or refute the results. The numbers recruited for this study represent the maximum number available at the time of the study; however, prospective sampling has been initiated. The discordant twin pairs were selected in the previous study because the index twin had been diagnosed >5 years prior, so the risk of diabetes developing in the nondiabetic twin was less than 10% (19). In the present study, we selected discordant pairs in which the index twin had been diagnosed for more >11 years, and the nondiabetic twin had normal glucose tolerance and no diabetes-associated autoantibodies. In the present study, the risk of the nondiabetic twins developing diabetes is calculated as <2% (16,19). The more precise identification of twin pairs likely to remain discordant for diabetes is an important feature of this present study. Comparison of our discordant twins with a previous study of an ICA-negative control population (n = 63) using the same methodology (24) confirms the importance of HLA-DQ in the susceptibility to type 1 diabetes in these twins. There was an increase in DQB1*0302 in discordant twins (65% positive) compared with controls (15.9% positive; P = 0.0004) and a decrease in the protective DQB1*0602/3 allele (5.4% positive compared with 30.2%; P = 0.003). This argues against a purely environmental cause for diabetes, even in twins discordant for disease. Last, the HLA-DR study of 1983 and the current HLA-DQB1 study are not directly comparable with regard to HLA class II typing. Although both HLA-DQB1*0201 and DQB1*0302 are in linkage disequilibrium with HLA-DR3 and DR4, respectively, they also form haplotypes with other DR antigens.

No twin studies have assessed the impact of non-HLA genes on susceptibility to type 1 diabetes. We found an increased frequency of diabetes-associated insulin genotypes in the concordant twins. The insulin gene is a plausible candidate gene for type 1 diabetes because of its β-cell–specific expression. It has been suggested that differences in the insulin gene

transcription could depend on sequence variation in the disease-associated hypervariable region, although different studies have revealed discrepant results, depending on the reporter construct and the in vivo/vitro models used (25–30). Interestingly, whereas twins concordant for diabetes had a higher frequency of disease-associated insulin genotypes than discordant pairs, twins discordant for diabetes did not have a greater frequency than did the islet cell–negative control population (31) (Table 2). This supports the hypothesis that there is a decreased load of diabetes susceptibility genes in discordant compared with concordant twins. Last, this study would also support the interaction of HLA-DQ and insulin gene alleles in determining susceptibility and protection to type 1 diabetes.

Acknowledgments— This study was supported by the Diabetic Twin Research Trust, the Joint Research Board of St. Bartholomew's Hospital, and the British Diabetic Association.

K.A.M. was a British Diabetic Association R.D. Lawrence Research fellow during this study.

We thank the twins and physicians who made these studies possible, as well as Dr. David Pyke, who initiated the twin study.

References

1. Kaprio J, Tuomilehto J, Koskenvuo M, Romanov K, Reunanen A, Eriksson J, Stengard J, Kesaniemi YA: Concordance for type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes mellitus in a population based cohort of twins in Finland. *Diabetologia* 35:1060–1067, 1993
2. Davies JL, Kawaguchi Y, Bennet ST, Copeman JB, Cordell HJ, Pritchard LE, Reed PW, Gough SC, Jenkins SC, Palmer SM: A genome-wide search for human type 1 diabetes susceptibility genes. *Nature* 371: 130–136, 1994
3. Lucassen AM, Julier C, Berressi J-P, Boitard C, Froguel P, Lathrop M, Bell JI: Susceptibility to insulin dependent diabetes mellitus maps to a 4.1kb segment of DNA spanning the insulin gene and associated VNTR. *Nat Genet* 4:305–310, 1993
4. Bell GI, Horita S, Karam JH: A polymorphic locus near the human insulin gene is associated with insulin-dependent diabetes mellitus. *Diabetes* 33:176–183, 1984
5. Hitman GA, Tarn AC, Winter RM: Type 1 (insulin dependent) diabetes and a highly variable locus close to the insulin gene on chromosome 11. *Diabetologia* 28:218–222, 1985
6. Bergholdt R, Karlsen AE, Johannesen J, Hansen PM, Dinarello CA, Nerup J, Pociot F: Danish Study Group of Diabetes in Childhood: characterisation of polymorphisms of an interleukin-1 receptor type 1 gene (IL1R1) promoter region (P2) and their relation to insulin-dependent diabetes mellitus (IDDM). *Cytokine* 7:727–728, 1995
7. Metcalfe KA, Hitman GA, Pociot F, Bergholdt R, Tuomilehto-Wolf E, Tuomilehto J, Viswanathan M, Ramachandran A, Nerup J, The DiMe Study Group: An association between type 1 diabetes and the interleukin-1 receptor type 1 gene. *Hum Immunol* 51:41–48, 1996
8. Marron MP, Raffel LJ, Garchon HJ, Jacob CO, Serrano-Rios M, Martinez Larrad MT, Teng WP, Park Y, Zhang ZX, Goldstein DR, Tao YW, Beaurain G, Bach JF, Huang HS, Luo DF, Zeidler A, Rotter JI, Yang MC, Modilevsky T, Maclaren NK, She JX: Insulin-dependent diabetes mellitus (IDDM) is associated with CTLA4 polymorphisms in multiple ethnic groups. *Hum Mol Genet* 6:1275–1282, 1997
9. Donner H, Rau H, Walfish PG, Braun J, Siegmund T, Finke R, Herwig J, Usadel KH, Badenhoop K: CTLA4 alanine-17 confers genetic susceptibility to Graves' disease and to type 1 diabetes mellitus. *J Clin Endocrinol Metab* 82:143–146, 1997
10. Nistico L, Buzzetti R, Pritchard LE, Van der Auwera B, Giovannini C, Bosi E, Larrad MT, Rios MS, Chow CC, Cockram CS, Jacobs K, Mijovic C, Bain SC, Barnett AH, Vandewalle CL, Schuit F, Gorus FK, Tosi R, Pozzilli P, Todd JA: The CTLA-4 gene region of chromosome 2q33 is linked to, and associated with, type 1 diabetes. *Hum Mol Genet* 5:1075–1080, 1996
11. McDermott MF, Ramachandran A, Ogunkolade BW, Aganna E, Curtis D, Boucher BJ, Snehalatha C, Hitman GA: Allelic variation in the vitamin D receptor influences susceptibility to IDDM in Indian Asians. *Diabetologia* 40:971–975, 1997
12. Pani MA, Knapp M, Donner H, Braun J, Baur MP, Usadel KH, Badenhoop K: Vitamin D receptor allele combinations influence genetic susceptibility to type 1 diabetes in Germans. *Diabetes* 49:504–507, 2000
13. Fava D, Gardner S, Pyke DA, Leslie RDG: Evidence that the age at diagnosis to IDDM is genetically determined. *Diabetes Care* 21:925–929, 1998
14. Johnston C, Pyke DA, Cudworth AG, Wolf E: HLA-DR typing in identical twins with insulin dependent diabetes: a difference between concordant and discordant pairs. *BMJ* 286:253–255, 1983
15. Verge CF, Gianani R, Yu L, Pietropaolo M, Smith T, Jackson RA, Soeldner S, Eisenbarth GS: Late progression to diabetes and evidence for chronic β-cell autoimmunity in identical twins of patients with type 1 diabetes. *Diabetes* 44:1176–1179, 1995
16. Tun RYM, Peakman M, Alviggi L, Hussain MJ, Lo SS, Shattock M, Pyke DA, Bottazzo GF, Vergani D, Leslie RD: Importance of persistent cellular and humoral immune changes before diabetes develops: prospective study of identical twins. *BMJ* 308: 1063–1068, 1994
17. Hawa M, Rowe R, Lan MS, Notkins AL, Pozzilli P, Christie MR, Leslie RD: Value of antibodies to islet protein tyrosine phosphatase-like molecule in predicting type 1 diabetes. *Diabetes* 48:1270–1275, 1997
18. Barnett AH, Eff C, Leslie RDG, Pyke DA: Diabetes in identical twins. *Diabetologia* 20:87–93, 1981
19. Olmos P, A'Hern R, Heaton DA, Millward BA, Risley D, Pyke DA, Leslie RD: The significance in the concordance rate for type 1 (insulin-dependent) diabetes in identical twins. *Diabetelogia* 31:747–750, 1988
20. National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories with glucose tolerance. *Diabetes* 28:1039–1057, 1979
21. Schmidli RS, Colman PG, Bonifacio E: Disease sensitivity and specificity of 52 assays for glutamic acid decarboxylase antibodies: the Second International GADAB Workshop. *Diabetes* 44:636–640, 1995
22. Nomura N, Ota M, Tsuji K, Inoko H: HLA-DQB1 genotyping by a modified PCR-RFLP method combined with group-specific primers. *Tissue Antigens* 38:53–59, 1991
23. Balnaves ME, Nasioulas S, Dahl H-HM, Forrest S: Direct PCR from CVS and blood lysates for detection of cystic fibrosis and Duchenne muscular dystrophy deletions. *Nucleic Acids Res* 19:115, 1991
24. Fennessy M, Hitman GA, Moore RH, Metcalfe K, Medcraft J, Sinico RA, Mustonen JT, D'Amico G: HLA-DQ gene polymorphism in primary IgA nephropathy in three European populations. *Kidney Int* 49:477–480, 1996
25. Bennett ST, Lucassen AM, Gough SC, Powell EE, Undlien DE, Pritchard LE, Merriman ME, Kawaguchi Y, Dronsfield MJ, Pociot F, Nerup J, Bouzekri N, Cambon-Thomsen A, Ronningen KS, Barnett AH, Bain SC, Todd JA: Susceptibility to human type 1 diabetes at IDDM2 is determined by tandem repeat variation at the insulin gene minisatellite locus. *Nat Genet* 9:284–292, 1995
26. Kennedy CG, German MS, Rutter WJ: The minisatellite in the diabetes susceptibility locus IDDM2 regulates insulin transcription. *Nat Genet* 9:293–298, 1995
27. Lucassen AM, Screaton GR, Julier C, Elliot TJ, Lathrop M, Bell JI: Regulation of insulin gene expression by the IDDM associated, insulin locus haplotype. *Hum Mol*

Genet 4:501–506, 1995
28. Owerbach D, Gabay KH: The search for IDDM susceptibility genes: the next generation. *Diabetes* 45:544–551, 1996
29. Pugliese A, Zeller M, Fernandez A Jr, Zalcberg LJ, Bartlett RJ, Ricardi C, Pietropaolo M, Eisenbarth GS, Bennett ST, Patel DD: The insulin gene is transcribed in the human thymus and transcription levels correlated with allelic variation at the INS VNTR-IDDM 2 susceptibility locus for type 1 diabetes. *Nat Genet* 15:293–297, 1997
30. Vafiadis P, Bennett ST, Todd JA, Nadeau J, Grabs R, Goodyer CG, Wickramasinghe S, Colle E, Polychronakos C: Insulin expression in human thymus is modulated by INS VNTR alleles at the IDDM2 locus. *Nat Genet* 15:289–292, 1997
31. Siddiqi A, Metcalfe KA, Lai M, Bingley PJ, Sachs J, Bottazzo GF, Gale EAM, Hitman GA: Susceptibility loci in ICA positive subjects, IDDM and other auto immune disease: implications for disease prediction. *Diabetologia* 37 (Suppl. 1):A55, 1994