ARTICLE

Estimation of Genetic Risk for Type 1 Diabetes

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The most important gene loci defining risk of type 1 diabetes mellitus (T1DM) are located within the HLA gene region. HLA-DQ molecules are of primary importance but HLA-DR gene products modify the risk conferred by HLA-DQ. The risk associated with an HLA genotype is defined by the particular combination of susceptible and protective alleles. The highest risk is associated with a combination of two different risk haplotypes (7% risk to develop T1DM in Finland) whereas protective genotypes covering 69% of population have a risk of less than 0.2%). The complicated analysis of HLA genotypes is simplified by strong linkage disequilibrium between HLA-DRB1, -DQA1 and -DQB1 loci. In many cases one can deduce the alleles of other loci based on determination of the alleles in one locus. Differences between various populations in the frequency of marker alleles and in the linkages between them has to be taken into account. We have developed PCR based typing methods that utilize blood spot samples, microtiter plate format and lanthanide labeled oligonucleotide probes to define HLA-DQ and -DR alleles relevant for T1DM risk. Typing is run stepwise so that after initial HLA-DQB1 typing only those samples will be further analyzed in which -DQA1 or -DRB1 typing is informative and expected to contribute to the risk estimation. This method has been used to screen more than 50,000 newborn infants in Finland over a time period of 6 years, and it has been able to identify most children who have developed T1D during the follow-up period. The efficiency of the procedure has also been tested in Finnish and Greek populations.

KEY WORDS: type 1 diabetes; genetic risk; HLA genes; risk estimation; genetic screening

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INTRODUCTION

It is well known that the susceptibility to develop type 1 diabetes mellitus (T1DM) is inheritable. This is also reflected by the familial aggregation of the disease. The most important genes defining the risk for T1DM are located within the HLA gene complex and they are responsible for about half of the total genetic component [Vyse and Todd, 1996]. The remaining genetic susceptibility is conferred by a large number of loci, each one with a minor effect. Among the genes outside the HLA region only the effect of polymorphism within the insulin gene region has been firmly established, whereas others have been only preliminarily localized [Cox et al., 2001].

The susceptibility associated with the HLA region is also complex. The prevailing view is that HLA-DQ heterodimer molecules encoded by HLA-DQA1 and -DQB1 genes are the major determinants of the disease risk. The high polymorphism of these genes and the existence of molecules associated with different degree of risk or dominant protection complicate the risk assessment. HLA-DQ does not alone define the risk, because HLA-DR molecules have a modifying effect [She, 1996], and still some authors have recently suggested that the risk can be best defined by HLA-DR typing alone [Zamani et al., 1996].

In addition to HLA-DQ and -DR loci there is evidence that several other HLA regions contribute to the risk. The third Class II locus, HLA-DP seems to have a small effect [Noble et al., 2000], and also genes in the Class I region appear to be of importance. Loci within the TNF region as well as a locus telomeric to Class I close to the microsatellite marker D6S2223 have been reported to affect the disease susceptibility [Moghaddam et al., 1998; Lie et al., 1999]. In parallel a major effect of the H2-A locus, homologous with human HLA-DQ has been demonstrated in the NOD mouse model of autoimmune diabetes as well as the modification of the disease risk by the H2-E locus homologous to HLA-DR [Lund et al., 1990; Ikegami et al., 1995]. This analogy further extends to the contribution of Class I loci in the mouse model.

TABLE I. HLA-DQ Molecules Associated With Susceptibility to or Protection From Type 1 Diabetes

Genes	Haplotype (*cis*) or genotype (*trans*)
Encoding risk associated molecules	
DQA1*0301-DQB1*0302	DR4-DQ8
DQA1*0501-DQB1*0201	DR3-DQ2
DQA1*0301-DQB1*0201	DR4-DQ2 (Mediterranean), DR7-DQ2 (Black), DR3-DQ2/DR4-DQ8
Encoding protective molecules	
DQA1*0101-DQB1*0602	DR15(2)-DQ6
DQA1*0501-DQB1*0301	DR5-DQ7
DQA1*0301-DQB1*0301	DR4-DQ7

The polypeptide chains of the HLA-DQ molecules are usually encoded by DQA1 and DQB1 genes in the same chromosome, in *cis* position, but may in some cases also be products of genes in different parental chromosomes, encoded in *trans* position. The very high risk associated with DR3-DQ2/DR4-DQ8 or DQA1*0501-DQB1*0201/DQA1*0301-DQB1*0302 genotype may thus be associated with the fact that also DQA1*0301 and DQB1*0201 in *trans* position are encoding a specific heterodimer. This heterodimer is also found in *cis* in the black DR7 haplotype and in the Mediterranean DR4 (DRB1*0405) haplotype.

The HLA-DQ molecules associated with increased risk of or protection against the disease are listed in Table I. Because of the very strong linkage between HLA-DQA1 and DQB1 loci it is in fact necessary in most cases to define only the DQB1 allele, and the DQA1 allele can be deduced based on the common linkage disequilibrium although some notable exceptions from this rule do exist.

RISK CALCULATION BASED ON HLA-DQ GENOTYPE

The importance of HLA-DQ molecules for diabetes risk is well discerned, when the frequency of different HLA-DQB1 genotypes is compared between children with T1D and the background population. Various measures of risk are used such as the odds ratio (OR) representing the ratio between the risk in subjects with and without a certain genotype. One can also calculate the positive predictive value (PPV) that takes into account the prevalence of the disease in the population and reflects the probability of a newborn infant with that genotype to develop the disease. A simple HLA-DQB1 typing that defines the presence of the HLA-DQB1*02 and DQB1*0302 alleles associated with risk as well as protection associated DQB1*0301, DQB1*0602, and DQB1*0603 alleles already produces a substantial number of genotypes (Table II), each one with a characteristic risk of its own. There is a gradient from very high risk associated with the genotype with two risk alleles to strong protection associated with genotypes with protective alleles alone or together with neutral alleles. The hierarchy among risk and protection associated alleles is clearly seen e.g., in the dominant protection associated with the DQB1*0602 allele. Even the DQB1*0302/*0602 genotype, a combination of a high risk and a strongly protective allele is very rare among diabetic children but DQB1* 0301/*0302 and DQB1*0302/*0603 genotypes combining the same strong risk allele with an allele

Dominant protection to type 1 Diabetes is associated with the DQB1*0602 allele

TABLE II. HLA-DQB1 Genotypes Among Finnish Children With Type 1 Diabetes (T1D) and Healthy Newborn Infants

	Children with T1D		Newborn infants			
HLA-DQB1* genotype	*n*	%	*n*	%	OR	PPV
02/0302	161	28.8	303	2.9	13.63	7.03
0302	191	34.1	1039	9.9	4.73	2.55
0302/0603	17	3.0	213	2.0	1.52	1.12
0301/0302	23	4.1	293	2.8	1.50	1.10
02	102	18.2	1384	13.1	1.47	1.04
02/0301	9	1.6	321	3.0	0.52	0.40
302/0602	6	1.1	324	3.1	0.34	0.26
Others	27	4.8	1704	16.2	0.26	0.22
02/0603	4	0.7	273	2.6	0.27	0.21
0301	10	1.8	1049	10.0	0.16	0.14
02/0602	2	0.4	406	3.9	0.09	0.07
0301/0603	1	0.2	198	1.9	0.09	0.07
0602	4	0.7	1421	13.5	0.05	0.04
0602 or 0603	3	0.5	1264	12.0	0.04	0.03
0301/0602	0	0.0	349	3.3	0.00	0.00
	560		10541			

The positive predictive value (PPV) is calculated based on a risk of 0.75% to develop childhood type 1 diabetes.

of lesser degree of protection are more common and in fact associated with disease risk. These weaker protective markers can instead easily protect against the disease risk conferred by the weaker risk allele DQB1*02, which alone is associated with a low disease risk.

*DQB1*02 and DQB1*0302 are associated with disease risk*

For practical purposes this type of grading system can be simplified for example by grouping all genotypes with a risk clearly smaller than that in the general population. Odds ratio can also be calculated against this group comprising the vast majority (69%) of the population. The risk of subjects with the high-risk HLA-DQB1*02/*0302 genotype is in fact 58 times that of this population majority.

The same HLA-DQ molecules are associated with diabetes risk in various Caucasian and black populations although their relative frequency in background populations varies, which is also reflected in genotypes found among type 1 diabetes patients. The comparison of frequencies of HLA-DQ genes associated with risk or protection between Greek and Finnish children with T1DM and newborn infants representing the background populations is shown in Table III. Finland and Greece represent the highest and the lowest incidence of type 1 diabetes countries, being on the extreme north and south of the Europe [Green et al., 1992]. In both populations DQB1*02 and DQB1*0302 are associated with disease risk and DQB1*0602/3 and DQB1*0301 with protection, but most Finnish children with T1DM are positive for DQB1*0302, whereas in Greece the DQB1*02 is the risk allele observed in most cases. It is tempting to hypothesize that the high frequency of the protective DQB1*0301 allele as well as the low frequency of DQB1*0302 in Greece might contribute to the lower disease incidence there. This relationship, however, is not so simple as the strongly protective DQB1*0602 allele is also common in Finland as well as in other Northern European countries, whereas it is rare in Greece and many other Mediterranean countries.

Effect of HLA-DQA1 and -DR4 Typing

Both HLA-DQB1 and -DQA1 genes are polymorphic and affect the conformation of the molecule and especially the peptide binding groove. These polymorphisms define which peptide will be bound and presented to T cells thus shaping the T cell repertoire. This variability is the most likely mechanism behind the positive and negative disease associations of specific HLA Class II molecules including the associations with type 1 diabetes [Thorsby, 1997].

We stated earlier that because of the strong linkage disequilibrium between DQB1 and DQA1 genes one can in most cases deduce the allele in the DQA1 locus if the DQB1 allele is known. Accordingly, when the HLA-DQB1*0302 allele is present, one can conclude that it is associated with DQA1*0301, and the complete susceptibility molecule is present. Similarly

TABLE III. Comparison of the Frequencies of HLA-DQB1 Alleles Associated With Risk for and Protection From Type 1 Diabetes in Greece and Finland

	Patients		Controls			
HLA-DQB1 allele	*n*	%	*n*	%	*P*	OR
Greek						
02	81	68.1	293	28.0	< 0.0001	5.49
0301	11	9.2	557	53.2	< 0.0001	0.09
0302	48	40.3	113	10.8	< 0.0001	5.59
0602 or 0603	1	0.8	150	14.3	< 0.0001	0.051
	119		1047			
Finnish						
02	278	49.6	2688	25.5	< 0.0001	2.88
0301	43	7.7	2210	21.0	< 0.0001	0.31
0302	398	71.1	2172	20.6	< 0.0001	9.47
0602 or 0603	37	6.6	4448	42.2	< 0.0001	0.10
	560		10541			

DQB1*0602 is practically always associated with DQA1*0102 but also with the DRB1*15 and DRB5*0101 alleles. Additional typing does not produce any useful information in the case of this strongly protective haplotype. HLA-DQB1*0301 may be associated with either DQA1*0301 (the DR4 haplotype) or DQA1*0501 (the DR5 haplotype and the DR6 haplotype). None of these is associated with diabetes risk, although there may be differences in the strength of protection. The value of DQA1 typing in this case remains of negligible practical value. Instead it is important to define the DQA1 allele associated with DQB1*02. In Northern European Caucasians DQB1*02 is associated with either DQA1*0201 (the DR7 haplotype that is protective or neutral) or DQA1*0501 (the DR3 haplotype associated with diabetes risk). In Mediterranean countries and in black populations it may also be associated with DQA1*0301 and is in both cases associated with diabetes risk, although the haplotype is very different for the DR locus, either DR7 or DR4 (DRB1*0405).

The major effect contributed by the HLA-DR locus is seen in the variability of diabetes-risk conferred by HLA-DR4 positive HLA-DQB1*0302 haplotypes depending on the specific DR4 subtype. HLA-DRB1*04 can now be divided into 42 different alleles starting from HLA-DRB1*0401–0442 of which only the first eight alleles from HLA-DRB1*0401–0408 are commonly defined. The HLA-DRB1*0403 allele present in Caucasian populations as well as the very similar DRB1*0406 allele found in Orientals are strongly protective according to several studies. HLA-DRB1*0403 exerts a dominant protection even in the high risk DR3-DQ2/DR4-DQ8 genotype [Vander-Auwera et al., 1995]. HLA-DRB1*0401, *0402, *0404, and *0405 are instead all associated with disease risk [She, 1996]. The risk associated with DRB1*0404 may be a lower than that conferred by the other DRB1*04 risk alleles, but it is affected by polymorphisms within the Class I region [Nejentsev et al., 1997; Reijonen et al., 1997].

TYPING METHODOLOGY

There is a huge number of methods available for definition of HLA alleles associated with diabetes risk. Various DNA hybridization techniques have replaced serology and lymphocyte culture based methods used earlier. A multitude of methods are also used for molecular typing and the development of these techniques is rapidly progressing. Restriction fragment length polymorphisms analyzed with long radioactive cDNA probes were originally used for genome based typing but were then replaced by PCR based methods. Future development may produce techniques that can handle enormous amounts of reactions without significant increase in analysis cost, but so far the number of loci and specific sequences to be analyzed are also important due to economic considerations.

The results presented in this study have been produced using a hybridization assay based on microtiter plates and lanthanide labeled probes developed for screening of selected HLA alleles. This methodology was developed during the mid 1990s and has been applied in the Finnish Diabetes Prediction and Prevention project [Kupila et al., 2001]. The format based on microtiter plates allows easy handling that can also be automated. The laborious extraction of DNA is avoided by using blood spots on filter paper of which a small piece is punched directly into the amplification mixture in microtiter plate wells. One of the HLA-DQB1 specific primers is biotinylated and amplicons transferred to streptavidin coated plates will thus be bound to the wells. After denaturation a hybridization mixture containing three sequence specific probes for each well is

added. Labeling of the probes with different lanthanide chelates allows simultaneous measurement of hybridization reactions based on different properties (time delay and wawe length) of these chelates. The original system of two mixes able to define the HLA-DQB1*02, *0301, *0302, and *0602/3 specificities [Sjöroos et al., 1995] was later further developed to differentiate between DQB1*0602 and *0603 as well as applied to HLA-DQA typing (a mix of DQA1*0201, *03, and *05 specific probes) and HLA-DR4 subtyping (two mixes defining DQRB1*0401, *0402, *0403/6, *0404, *0405, *0407, and *0408) [Nejentsev et al., 1999].

In the Finnish Diabetes Prediction and Prevention (DIPP) study more than 50,000 newborn infants have been screened for T1D associated genotypes. Prospective observation has demonstrated that more than 70% of the first 50 children who progressed to clinical T1D from among the screened cohorts were correctly classified according to the genetic screening. This result actually exceeds the expected efficiency based on estimations done on genotype frequencies among affected children, probably due to the increased frequency of HLA risk gene alleles in children developing the disease at a very early age [Komulainen et al., 1999].

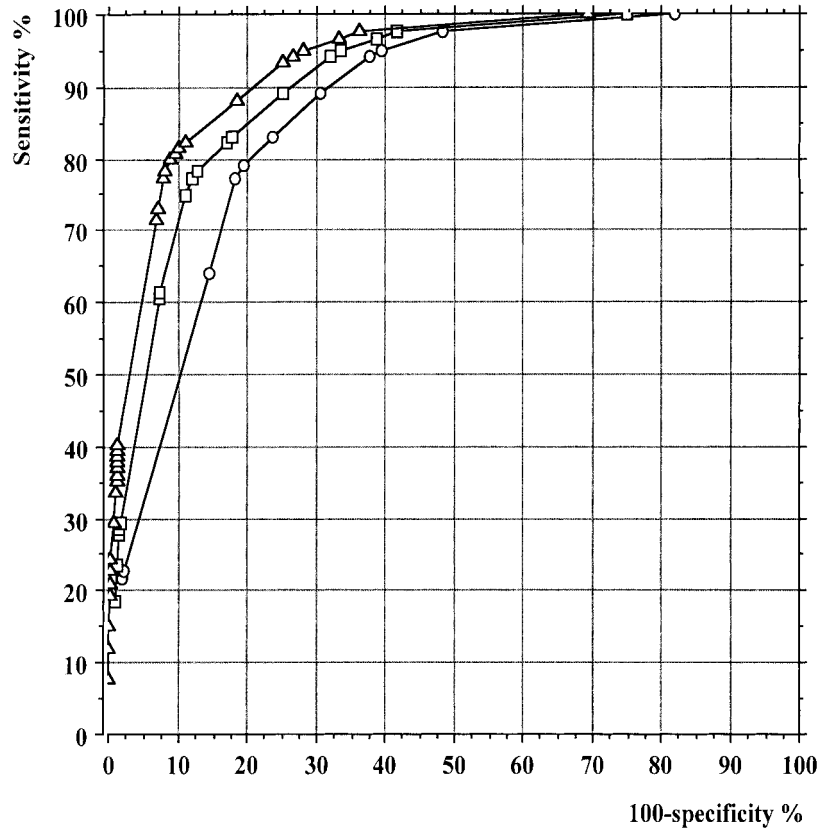

Figure 1. The sensitivity and specificity of various HLA based genetic screening strategies for type 1 diabetes in the Greek population. (○) HLA-DQB1 typing (five alleles). (□) Additional HLA-DQA1 typing in DQB1*02 positives subjects (three alleles). (△) Additional DR4 subtyping (6 alleles) in DQB1*0302 positive subjects.

USE OF RISK ESTIMATION AND TYPING STRATEGY

So far, there is no preventive treatment of type 1 diabetes and the screening of genetic susceptibility is mainly indicated in the framework of specific research projects. Among families with one or more affected members one often meets, however, requests for information concerning on the genetic risk of unaffected family members, especially that of sibs of a child with T1DM. The knowledge of a low genetic risk in the sibs may be comforting for the parents in some cases. When estimating the disease risk of siblings one has to take into account the considerably higher prevalence of the disease in first-degree relatives but also the common presence of risk alleles in the genotypes of unaffected family members. Familial aggregation of the disease seems to be associated to some extent with accumulation of HLA risk genotypes. A comparative study in Finland showed that familial cases had more often high-risk associated HLA genotypes than sporadic cases [Veijola et al., 1996].

The main purpose of genetic screening remains the enrichment of subjects at genetic risk for various research projects, either prevention trials or etiological studies. If a prevention trial is based on an absolute safe and cheap treatment modality one can apply it to large cohort without any screening, but if the costs are considerably high or there are possible risks associated with the treatment, one has to identify subjects with as high risk as possible. Because the beta-cell destructive autoimmune process usually starts years before clinical disease, etiological studies aimed at identifying environmental triggers need extensive follow-up with frequent sample collection and the selection of subjects with high genetic risk is of apparent advantage. Secondary prevention trials are targeted on subjects positive for diabetes-associated autoantibodies. Also these subjects can most efficiently and in an early phase of the process be identified by testing frequently for autoantibodies subjects who have been identified to be at increased risk by genetic screening.

The efficiency of genetic risk estimation in terms of sensitivity and specificity is presented in Figure 1. Because of the graded risk associated with various genotypes this can be best expressed by a cumulative curve where sensitivity increases when new genotypes conferring smaller risk are included in the risk group. A reciprocal decrease of specificity is naturally occur-

ring. The specificity is expressed on the x-axis as 100%-specificity reflecting the proportion of the background population in which the proportion of cases indicated by sensitivity is detected. The starting point of the curve identifies the small group of very high risk individuals. This group of heterozygotes with two high risk haplotypes have a very low frequency among the general population but a considerable proportion of children with T1DM are covered. By gradual inclusion of genotypes with lesser risk one can reach a sensitivity of more than 90%, although the specificity of the test is then very poor, about half of the general population carrying the genotypes being included. For various purposes different strategies of screening can be selected. The possibilities range from targeting the small group of children at the highest risk for frequent sampling to a relatively extensive series to be able to identify most of the children eventually developing T1DM.

The efficiency of the screening can be increased by adding new loci and markers. It appears in Figure 1 how the curve is moving to the left when initially HLA-DQA1 and thereafter HLA-DR4 subtyping is added to the screening protocol. This means increased sensitivity and specificity when more tests are used. The cost of additional tests can be considerably reduced by a stepwise typing strategy in which these tests are performed only in those samples in which they will be informative based on the HLA-DQB1 typing results.

RISK ASSOCIATED WITH EACH MARKER IN DIFFERENT POPULATIONS: AETIOLOGIC CONSIDERATIONS

The population to which the screening protocol will be applied has to be considered when selecting the markers to be used. As earlier stated the same markers are associated with disease risk and protection in most populations, but the frequency of the markers may be very different in the background population. Thus in Finland more than 60% of the children with T1DM are positive for either the DQB1*02/DQB1*0302 or DQB1*0302/x (x $\neq$ *02,*0301, *0602 or *0603) genotypes, and these two genotypes were originally selected in the population based screening program. Due to the low frequency of DQB1*0302 in Greece these two genotypes cover only 36% of the affected children. The inclusion of DQB1*02 positive genotypes is accordingly important when identifying children at genetic diabetes risk in Greece. Figure 1 showing the increase in screening efficiency by additional DQA1 and DR4 typings is based on analyses done in the Greek population. DQA1 typing differentiating between the protective DQA1*0201 (DR7) allele and the susceptibility alleles DQA1*05 (DR3) and DQA1*03 (DR4) is important among those with the DQB1*02 haplotypes because all these haplotypes are common in the Greek population. DR4 subtyping in DQB1*0302 positive haplotypes is also essential, because the frequency of the protective DRB1*0403 allele is high in those haplotypes. In Finland the population is much more homogeneous, DQB1*02 is in most cases associated with DQA1*05 and DQB1*0302 is in the vast majority of cases either with DRB1*0401 or with DRB1*0404, whereas the protective DRB1*0403 allele is rare in the background population [Nejentsev et al., 1999]. The benefit of these additional typings in terms of test sensitivity and specificity is accordingly quite low in the Finnish population.

For an individual, however, the full information might still be important. Table IV shows the relative risk associated with the high risk DQB1*02/*0302 genotype when additional DQA1 and DRB1*04 typings are performed and the result shows the presence of higher or lower risk. Thus the child with both DRB1*0403 and DQA1*0201has in fact a low risk to develop diabetes even if only very few of the children with high risk DQB1*02/*0302 have this protective genotype.

Although the same haplotypes are associated with risk and protection in various populations the strength of association may still be different. The DQA1*05-DQB1*02 (DR3) haplotype is more common in Greek than in Finnish population and it also seems to be associated with a higher risk in Greece. Table V shows the different OR values in the population according to a similar risk genotype. The difference

TABLE IV. Risk Associated With the HLA-DQB1*02/*0302 Genotype in Finland When Additional Typing for DQA1 and DRB1*04 Alleles Detects Alleles Conferring Either High or Low Risk

	Diabetic children $n = 560$		Newborns $n = 10541$			
	n	%	*n*	%	RR	PPV
Increasing risk						
HLA-DQB1*02/*0302	161	28.75	303	2.87	13.63	7.03
with HLA-DQA1*05-DQB1*02	144	25.63	224	2.12	15.88	8.36
and HLA-DRB1*0401-DQB1*0302	111	19.86	156	1.48	16.52	9.22
Decreasing risk						
HLA-DQB1*02/*0302	161	28.75	303	2.87	13.63	7.03
with HLA-DQA1*0201-DQB1*02	17	3.12	79	0.75	4.26	3.05
with HLA-DRB1*0403-DQB1*0302	2	0.35	13	0.12	2.78	2.05

TABLE V. Risk Conferred by HLA-DQA1*05-DQB1*02/y Genotype in Finland and Greece

	Children with T1D	Unselected newborn infants	
Country	*n* (%)	*n* (%)	OR (95% CI)
Finland	53/316 (16.8)	88/1000 (8.8)	2.09 (1.42–3.06)
Greece	37/119 (31.1)	58/1047 (5.5)	7.69 (4.68–12.83)

did not change when DQB1*0302 positive haplotypes were removed from the calculation. Whether this difference between Greek and Finnish DR haplotypes reflects the effect of additional genetic factors as demonstrated in DR3-positive haplotypes, e.g., in high incidence Sardinia [Zavattari et al., 2001] or is caused by different environmental interactions in Northern and Southern European populations remains to be defined.

CONCLUSIONS

In conclusion, HLA typing can be applied for estimating the genetic risk of T1D in various populations. The accuracy of the estimation can be increased by including multiple loci into the protocol. The main purpose of the risk estimation is at the moment the identification of subjects at high risk for research studies on the etiology and the natural course of the disease process, and increasingly also for therapeutic trials. The efficiency of various markers in the screening protocol is highly dependent on the population to be studied.

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REFERENCES

Cox NJ, Wapelhorst B, Morrison VA, Johnson L, Pinchuk L, Spielman RS, Todd JA, Concannon P. 2001. Seven regions of the genome show evidence of linkage to type 1 diabetes in a consensus analysis of 767 multiplex families. Am J Hum Genet 69:820–830.

Green A, Gale EAM, Patterson CC for the EURODIAB ACE Study Group. 1992. Incidence of childhood-onset insulin-dependent diabetes mellitus: the EURODIAB ACE study. Lancet 339:905–909.

Ikegami H, Makino S, Yamato E, Kawaguchi Y, Ueda H, Sakamoto T, Takekawa K, Ogihara T. 1995. Identification of a new susceptibility locus for insulin-dependent diabetes mellitus by ancestral haplotype congenic mapping. J Clin Invest 96:1936–1942.

Komulainen J, Kulmala P, Savola K, Lounamaa R, Ilonen J, Reijonen H, Knip M, Akerblom HK. 1999. Clinical, autoimmune, and genetic characteristics of very young children with type 1 diabetes. Childhood Diabetes in Finland (DiMe) Study Group. Diabetes Care 22:1950–1955.

Kupila A, Muona P, Simell T, Arvilommi H, Savolainen H, Hämäläinen A-M, Korhonen S, Kimpimäki T, Sjöroos M, Ilonen J, Knip M, Simell O. 2001. Feasibility of genetic and immunological prediction of type 1 diabetes in a population-based birth cohort. Diabetologia 44:290–297.

Lie BA, Todd JA, Pociot F, Nerup J, Akselsen HE, Joner G, Dahl Jorgensen K, Ronningen KS, Thorsby E, Undlien DE. 1999. The predisposition to type 1 diabetes linked to the human leukocyte antigen complex includes at least one non-Class II gene. Am J Hum Genet 64:793–800.

Lund T, O'Reilly L, Hutchings P, Kanagawa O, Simpson E, Gravely R, Chandler P, Dyson J, Picard JK, Edwards A, Kioussis D, Cooke A. 1990. Prevention of insulin-dependent diabetes mellitus in non-obese diabetic mice by transgenes encoding modified I-A β-chain or normal I-E α-chain. Nature 345:727–729.

Moghaddam PH, deKnijf P, Roep BO, Vander-Auwera B, Naipal A, Gorus F, Schuit F, Giphart MJ. 1998. Genetic structure of IDDM1: Two separate regions in the major histocompatibility complex contribute to susceptibility or protection. Diabetes 47: 263–269.

Nejentsev S, Reijonen H, Adojaan B, Kovalchuk L, Sochnevs A, Schwartz EI, Åkerblom HK, Ilonen J. 1997. The effect of HLA-B allele on the IDDM risk defined by DRB1*04 subtypes and DQB1*0302. Diabetes 46: 1888–1892.

Nejentsev S, Sjöroos M, Soukka T, Knip M, Simell O, Lövgren T, Ilonen J. 1999. Population based genetic screening for type 1 diabetes risk in Finland: selective genotyping of the markers in the HLA-DQB1, -DQA, and -DRB1 loci. Diabet Med 16: 985–992.

Noble JA, Valdes AM, Thomson G, Erlich HA. 2000. The HLA Class II locus DPB1 can influence susceptibility to type 1 diabetes. Diabetes 49:121–125.

Reijonen H, Nejentsev S, Tuokko J, Koskinen S, Tuomilehto-Wolf E, Åkerblom HK, Ilonen J, the Childhood Diabetes in Finland Study Group. 1997. HLA-DR4 subtype and -B alleles in DQB1*0302 positive haplotypes associated with IDDM. Eur J Immunogenet 24:357–363.

She J-X. 1996. Susceptibility to type I diabetes: HLA-DQ and DR revisited. Immunol Today 17:323–329.

Sjöroos M, Iitiä A, Ilonen J, Reijonen H, Lövgren T. 1995. Triple-label hybridization assay for type-1 diabetes-related HLA alleles. BioTechniques 18:870–877.

Thorsby E. 1997. Invited anniversary review: HLA associated diseases. Hum Immunol 53: 1–11.

VanderAuwera B, VanWaeyenberge C, Schuit F, Heimberg H, Vandewalle C, Gorus F, Flament J, Belgian Diabetes Registry. 1995. DRB1*0403 protects against IDDM in Caucasians with the high-risk heterozygous DQA1*0301-DQB1*0302/DQA1*0501-DQB1*0201 genotype. Diabetes 44: 527–530.

Veijola R, Reijonen H, Vähasalo P, Sabbah E, Kulmala P, Ilonen J, Åkerblom HK, Knip M. 1996. HLA-DQB1-defined genetic susceptibility, beta cell autoimmunity, and metabolic characteristics in familial and nonfamilial insulin-dependent diabetes mellitus. J Clin Invest 98:2489–2495.

Vyse TJ, Todd JA. 1996. Genetic analysis of autoimmune disease. Cell 85:311–318.

Zamani M, Pociot F, Spaepen M, Raeymaekers P, Nerup J, Cassiman JJ. 1996. Linkage and association of the HLA gene complex with IDDM in 81 Danish families: strong linkage between DR beta 1(Lys71+) and IDDM. J Med Genet 33:899–905.

Zavattari P, Lampis R, Motzo C, Loddo M, Mulargia A, Whalen M, Maioli M, Angius E, Todd JA, Cucca F. 2001. Conditional linkage disequilibrium analysis of a complex disease super locus, IDDM1 in the HLA region, reveals the presence of independent modifying gene effects influencing the type 1 diabetes risk encoded by the major HLA-DQB1, -DRB1 disease loci. Hum Mol Genet 10:881–889.