chi-square value of 5×10^{-4} (not significant). Comparison of the distribution of Cw7, B8, DR3, DQ2 haplotypes yields a chi-square value of 6×10^{-3} (not significant). If HLA-A really does contribute to diabetes susceptibility in addition to the other loci on the extended HLA haplotype, a much larger study would be required to demonstrate statistical significance.

The authors state correctly that a powerful method of analysing whether HLA-A alleles predispose to IDDM independently of class II alleles is to examine the distribution of class I alleles in those diabetic subjects who possess neither of the high-risk class II alleles encoding DR3 and DR4. The authors found that 47 of 55 diabetic subjects possess two copies of the high-risk HLA-A alleles compared with 40 of 58 control subjects, $\chi^2 = 4.33$, ($p < 0.05$). This weak association is interesting but the authors do not show the data for the remaining subjects who possess one or none high-risk HLA-A alleles. Unless there is any reason to suggest that high-risk HLA-A alleles predispose to IDDM recessively we believe that the entire distribution of high-risk A alleles should be examined before any

deductions concerning their effect on diabetes susceptibility in non-DR3/non-DR4 subjects can be made.

Although HLA class I genes are attractive candidate determinants of diabetes susceptibility, we believe that more data are required to test the hypothesis adequately. Given that the population studied by Fennessy et al. [1] is large and well-characterized, we suspect that alternative approaches need to be devised to determine whether different closely-related HLA loci make distinct contributions to susceptibility to IDDM in the Finnish population.

Yours sincerely,
D. Jenkins, C. Mijovic

Reference

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HLA-A associations with IDDM – a case of numbers?

Dear Sir,

The genetic risk for insulin-dependent diabetes mellitus (IDDM) conferred by the HLA class II genes cannot be entirely explained by differences at position 52 of the DQ α chain and position 57 of the DQ β chain. Risk conferred by different HLA-DQA1/B1 haplotypes with the same amino acids in these positions varies, as does the risk conferred by the same DQ haplotype occurring in combination with different HLA-DR alleles [1]. Several explanations have been given, including the influence of other linked genes [1, 2], and quantitative differences in HLA class II expression [3]. Fennessy et al. [4] suggest that the risk is influenced by gene(s) telomeric to HLA-C, at or in the vicinity of HLA-A. This region of the MHC has only recently been directly implicated in IDDM susceptibility [5].

This issue is addressed by analysing data from the Finnish cohort of IDDM families, the largest studied for HLA-A, -C, -B, -DR, -DQ haplotypes. They examine individual haplotypes differing only in the HLA-A region and show an association with IDDM only when certain HLA-A alleles are present. They also demonstrate a weak association of these HLA-A alleles with IDDM in non-DR3/non-DR4 patients and conclude that these HLA-A alleles modulate disease susceptibility. From a statistical standpoint, however, their conclusions cannot be supported. Although they show that the CW1 B56 DR4 DQ8 haplotype is significantly associated with IDDM when HLA-A2 is present on this haplotype, other HLA-A alleles are found too infrequently on this haplotype for statistical analysis, and association for these HLA-A alleles cannot be excluded. While the A2 containing haplotype was found on 96 occasions, there are only nine non-A2 CW1 B56 DR4 DQ8 haplotypes in their study, and clearly there is no statistical power to determine association in the non-A2 haplotypes. It cannot, therefore, be concluded that HLA-A2 confers sus-

ceptibility, nor that it increases the susceptibility conferred by CW1 B56 DR4 DQ8 alone. Indeed, if a statistical comparison is made between the risk conferred by A2 CW1 B56 DR4 DQ8 and non-A2 CW1 B56 DR4 DQ8 haplotypes, there is no significant difference for any of the haplotypes studied. The same is also true for the comparisons of HLA-A allele contribution to risk in the two most frequently found diabetic haplotypes in their population, CW3 B62 DR4 DQ8 and CW7 B8 DR3 DQ2. Statistically significant association with IDDM is seen only in haplotypes which are frequently represented, and again there is no difference between the risk conferred by haplotypes with different HLA-A alleles. The weak association of claimed susceptible HLA-A alleles with IDDM in non-DR3/non-DR4 individuals is also dubious since statistical significance is not maintained if a two-tailed chi-square analysis is performed. Moreover, while the authors claim large variation in genetic risk for IDDM for different DQ8 containing haplotypes in Finland [4, 6], none of those represented in this [4] and their previous report [6] have statistically different risks for IDDM.

One of the more remarkable findings of the HLA analysis of the Finnish DiMe study is the predominance of relatively few "conserved" HLA-A, -C, -B, -DR, -DQ haplotypes [6]. This conservation of haplotypes, emphasising the stability and low recombination within this stretch of DNA, complicates analyses of contributions to disease risk by separate loci. Fennessy and colleagues have attempted such an analysis, but even in the large DiMe study the high degree of polymorphism at each locus together with haplotype conservation has resulted in too few numbers for statistical comparison. Unfortunately, this is likely to be true for similar analyses at other loci within the MHC. While there are several good reasons why we should consider the importance of HLA class I in the genetic susceptibility and pathogenesis of IDDM, it appears unlikely that classic population-based studies will reach sufficient numbers to clarify a contribution to the genetic risk for IDDM by HLA-A. This region cannot yet be regarded as, nor excluded from, modulating IDDM susceptibility.

Yours sincerely,
E. Bonifacio

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Response from the authors

Dear Sir,

We thank Drs. Jenkins and Mijovic and Dr. Bonifacio for their comments regarding our paper [1]. Indeed, we agree that proving an independent effect of the HLA class I region compared to the class II region is a very difficult task rendered more complicated by the extensive linkage disequilibrium that exists between allelic variants of genes in the major histocompatibility complex (MHC). It was precisely for this reason that we did not rely on one experimental set, but several, to suggest that the class I region might modulate the risk contributed by the class II region to insulin-dependent diabetes mellitus (IDDM) susceptibility. The data sets were the analysis of the three main haplotypes (Cw1, Bw56, DR4, DQ8; Cw3, B62, DR4, DQ8 and Cw7, B8, DR3, DQ2), together with the further characterisation of the Bw56, DR4 haplotype, and the analysis of HLA-A alleles in the non-DR3, non-DR4 diabetic subjects and those unable to form a diabetes susceptibility DQ heterodimer. Each data set by itself is not conclusive but when combined they are strongly supportive of our hypothesis. The authors of both letters correctly point out the small number of non-A2, Bw56, DR4 haplotypes compared to A2, Bw56, DR4 haplotypes; it was for this reason we did not attempt to analyse the differences statistically. However, it remains that for the A2, Bw56, DR4 haplotype in IDDM subjects there is a selective increase maintained in the Finnish population over and above the other haplotypes with different A alleles (A2 vs non-A2 $\chi^2 = 38.7$, $p < 0.0001$). This would therefore imply that the conserved haplotypes associated with diabetes in the Finnish population include the HLA-A2 allele without necessarily implicating HLA-A2 itself. Similar arguments can be put forward for the other two main haplotypes. Furthermore, the three most frequent haplotypes (from HLA-A through to DQ) are present in 33 % of diabetic patients vs 10.3 % of control subjects. This hypothesis is then supported by the analysis of the non-DR3, non-DR4 diabetic subjects compared to control subjects in which certain HLA-A alleles are found more frequently than expected. Dr. Bonifacio

states that we should have used a two-tail chi-square test for the latter analysis. The statistics presented were based on a two-tailed Fisher's exact test as stated in the statistical section with a p value = 0.038; therefore, this is statistically significant.

Drs. Jenkins and Mijovic ask for a detailed breakdown of the non-DR3 non-DR4 subjects according to HLA-A alleles. In the diabetic probands 47, 8 and 0 subjects possessed two high-risk, one high-risk and no risk alleles, respectively; the corresponding numbers in the non-diabetic patients were 40, 17 and 1. Since the number of subjects with no high-risk HLA alleles is so low, we did not feel that a 3×2 chi-square comparison was appropriate. If two high-risk HLA-A alleles are required for susceptibility to IDDM, this does not necessarily imply an autosomal recessive model but could equally fit a co-dominant model; however, there is no agreement on the appropriate models of IDDM susceptibility in the literature.

In support of our overall hypothesis, and the use of such population based data, Demaine and colleagues [2] have also produced evidence to support an independent effect of the class I genes on IDDM susceptibility. This would therefore lend further support to our data and make it unlikely that we have described a false positive association. What our studies cannot address is whether the gene(s) that modulate the HLA class II effect is an HLA or a non-HLA gene. Several strands of evidence might suggest a role for HLA class I molecules in diabetes susceptibility as outlined in our paper. However, the primary association does not necessarily have to be with an HLA gene, which may merely be acting as a marker for a non-immune response gene involved in diabetes susceptibility. In order to identify such an IDDM susceptibility gene functional, not immunogenetic, studies will be required. We hope our studies, and those of others, will stimulate such experiments.

Yours sincerely,

M. Fennessy, K. Metcalfe, G. A. Hitman, P. A. Biro, J. Tuomilehto, E. Tuomilehto-Wolf

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