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Mating in parents of type 1 diabetes families as a function of the HLA DR-DQ haplotype

H. Kahles¹, O. Kordonouri², E. Ramos Lopez¹, M. Walter³, S. Rosinger³, B. O. Boehm³, K. Badenhop¹, C. Seidl⁴, and A. Ziegler⁵ the Type 1 Diabetes Genetics Consortium

¹Division of Endocrinology and Department of Immunohaematology, University Hospital Frankfurt, Frankfurt, Germany

²University Children's Hospital auf dem Buldt, Hannover, Germany

³Section Endocrinology, University Hospital Ulm, Ulm, Germany

⁴Red Cross Blood Transfusion Center, Institute of Transfusion Medicine and Immunohaematology, Frankfurt am Main, Germany

⁵Institute of Diabetes Research, University of Munich for the German Type 1 Diabetes Network (DT1DGN)

Abstract

Aim—The Major Histocompatibility Complex (MHC) region on chromosome 6p21 (*IDDM1*) contributes about half of the familial clustering of type 1 diabetes (T1D). Several studies have revealed that highly polymorphic genes within the MHC may associate with the mating choice. Our study should determine whether a specific mating effect is detectable in T1D families as a function of human leucocyte antigen (HLA) DR-DQ, which could contribute to disease susceptibility.

Methods—We analysed the parental HLA-DR genotypes in 829 diabetic families. The families derive from the Type 1 Diabetes Genetics Consortium (T1DGC) in addition to those of our own centre and the original UK, US and SCAND diabetic families.

Results—A total of 307 of 829 parental couples (37.0%) were matched for at least one known T1D risk haplotype (DR3 or DR4), which is significantly less than the expected 374.9 (45.2%), derived from population genotype frequencies ($p < 0.0009$). Parents share less susceptibility haplotypes and rather complement each other as both carry one different risk haplotype (DR3 or DR4). The number of such parental couples was significantly higher than expected (293 vs. 223.4; $p < 0.0003$). All non-transmitted DR haplotype pairs were also analysed. More often than expected, both parents did not transmit DR1 (94 vs. 59.1; $p < 0.003$) and DRy (y: not DR1, not DR3, not DR4; 63 vs. 30.3; $p < 0.0005$). In contrast, the parental non-transmitted pair of haplotypes DR1-DRy was observed to a far lesser extent than expected (26 vs. 84.7; $p < 10^{-8}$). These observations were only made in multiplex families, whereas in simplex families, no deviation from the expected frequencies was observed.

Conclusions—Our data are consistent with the conclusion that genes in the HLA region may influence the mating choice in parents of T1D patients, thus contributing to familial clustering of T1D in multiplex families. This may indicate a different parental background of multiplex compared with simplex T1D families.

Keywords

HLA class II; mating type; transmission; type 1 diabetes

Introduction

In 1976, Yamazaki *et al.* [1], for the first time, reported that individual-specific odours influence sexual selection in mice in a Major Histocompatibility Complex (MHC)-dependent way. Since then, several studies in vertebrates, including humans, have revealed that highly polymorphic genes within the MHC participate in mate choice. Therefore, besides their crucial function in adaptive and innate immune responses, the products of MHC genes also have a role in behavioural context, as well as in reproduction [2].

The connection between MHC and mate selection is presumably a consequence of the crucial importance of the MHC in immune responses, in which two classes of genotypes might be favoured: heterozygotes and rare alleles [3]. MHC heterozygotes have an advantage over homozygotes because more peptides can be represented to T cells and rare MHC alleles might prevent pathogen evasion of host immune responses or exhibit a lack of association with autoimmunity. These two mechanisms could select for MHC diversity but both would also lead to the evolution of preferences for mates that are MHC heterozygous and MHC distinct. The avoidance of inbreeding is an added benefit that can be a consequence of choosing an MHC heterozygous mating partner [4].

In Darwin's view, sexual selection was restricted to the precopulatory phase, during which the female selects the more agreeable partners. However, within the last decade, it has been realized that female choice operates during and after copulation as well. The mechanisms that influence mate choice in vertebrates have greatly been aided by the molecular identification of odorant receptors in 1991. There are three different types of chemosensory receptors within the nasal cavity of many vertebrates: olfactory receptors and two vomeronasal receptors (VR1 and VR2). Whereas olfactory receptor genes are expressed by olfactory neurones within the main olfactory epithelium (MOE), VR1 and VR2 are found on two types of neurones that are located in different zones of the vomeronasal organ (VNO) [5]. In contrast to the MOE, the VNO specializes in detecting small molecules and proteins (e.g., pheromones) that provide information about the sexual and social status of conspecifics. Stimulation of vomeronasal sensory neurones (VSNs) often initiates a behavioural or endocrine response. In humans, olfactory receptor genes occur on nearly all chromosomes, usually as clusters (e.g., the large olfactory receptor gene cluster linked to the human MHC). In addition, these different types of chemoreceptors not only fulfil their role of recognizing distinct ligands within the nose but are also used by spermatozoa as guidance cues. Possibly, their expression on spermatozoa enables a female to influence sperm motility through the establishment of chemical gradients within her reproductive tract. However, the recent demonstration that MHC peptides are the neuron-activating chemosignals has removed a major barrier to linking MHC genes with their associated behaviours.

In the mouse and human genomes, a large cluster of OR genes is tightly linked to the MHC. This fact, together with the high degree of linkage disequilibrium between the two types of loci in humans suggests the existence of a functional connection between the MHC and MHC-linked olfactory receptor loci [6]. Several attempts have already been made to identify the molecules involved in chemosignal-based communication but it is the study by Leinders-Zufall *et al.* [7] that shows unequivocally the influence of peptides that can be bound specifically by MHC class I antigens on the generation of odour types. These peptides must meet precise structural requirements to be effective, that is, they have to retain those amino acids that act as

anchors when presented by MHC class I molecules to activate the same VNO neurones [8]. VSNs responded in an MHC allele-specific manner with high sensitivity. Whereas substitution of peptide anchor residues with alanine resulted in the failure to stimulate these neurones, substitutions at non-anchor positions generally stimulated the same neurones [9].

These results point to the structural importance of peptide anchor residues in binding VR2 receptors and, given the similar binding properties of MHC molecules, reveal the similar ligand-binding properties of these unrelated molecules. More importantly, because peptides are ‘moulds’ of the antigen-binding site of MHC molecules, sensory receptors that detect peptides in an MHC-like fashion could, in effect, be an MHC genotyping system [9]. Another study demonstrates that MHC peptides alone are sufficient to convert the MHC odour type of an individual to that of a genetically dissimilar individual. These data experimentally confirm that MHC-presented peptides are odorants that control MHC-mediated behaviours. The following mouse experiments used class I peptides, whereas the stickleback experiments used both class I and II peptides [7,10,11]. When peptides from an MHC-dissimilar individual are added to odour sources that are MHC-similar to the responding individual, it increases the odour’s attractiveness in mice and in sticklebacks. These results indicate that peptides are sufficient to alter odour preferences in a fashion consistent with MHC-based mating preferences.

Even when a new male replaces the original mate of a recently mated female mouse, there is a high probability that the pregnancy will be aborted. Named after its discoverer, the Bruce effect works even if the original male is replaced by just the scent (e.g., urine) of a newmale, and the likelihood of abortion is maximized if the new male is MHC-disparate to the original male. Leinders-Zufall *et al.* [7] took advantage of this to show that MHC peptides could act alone as a signal of MHC genetic identity. Remarkably, addition of just three novel peptides to the male’s urine transformed low levels of pregnancy block into high levels. In other words, a few peptides provide a strong enough signal to convert an MHC-similar signal to an MHC-dissimilar one, resulting in a profound biological act – the destruction of one’s own embryos.

The analysis of mate preferences in different human populations is particularly complicated because religious, ethnic and social aspects have a role in reproductive behaviours. This results, for example, in a considerable degree of consanguinity in many parts of the world, and conflicting data were obtained with regard to an influence of the MHC on mate selection.

However, the human MHC also influences body odours and olfactory preferences that are not restricted to women but that can also be observed in men. Comparable studies in Hutterites, an isolated sect in North America, revealed that females exhibit a preference for paternally inherited human leucocyte antigen (HLA) alleles. Nevertheless, the preference for basic ingredients of perfumes also correlates with HLA type.

The MHC on chromosome 6p21 contributes about half of the familial clustering of type 1 diabetes (T1D) [12]. Our study should determine, whether a specific mating effect is detectable in T1D families as a function of HLA DR-DQ, which could contribute to disease susceptibility.

Materials and Methods

MHC class II haplotypes (DR-DQ) were provided by the T1DGC for 1815 couples of multiplex and 267 couples of simplex families. First, we calculated the HLA-DRB1 genotype frequencies in males and females separately and then analysed the existing mating constellation. The number of couples expected to match for a haplotype was calculated from parental genotype frequencies, assuming random parental mating habits.

Results

A total of 707 of 1815 parental couples (39.0%) of multiplex families matched for at least one known T1D risk haplotype (DR3 or DR4), which is significantly less than the expected 881.5 (48.6%), derived from overall parental genotype frequencies ($p = 6 \times 10^{-9}$; table 1). Parents of multiplex families seem to be characterized less by sharing susceptibility haplotypes than by complementing one another, as both carry one different risk haplotype (DR3 or DR4). The number of these parental couples was significantly higher than expected (667/1815 vs. 493.7/1815; $p = 9 \times 10^{-10}$). Within simplex families, no significant deviations from the expected frequencies were detectable. We also cannot find a significant difference in transmission rates of high-risk haplotypes between those two family groups (figure 1).

Discussion

T1D – as an autoimmune disease – might be driven by evolutionary mating habits preferring MHC heterozygosity and, thus, high-risk parental couples. Genes in the HLA region may influence the mating choice in parents of T1D, thus contributing to a familial clustering of T1D, especially in multiplex families. However, these observations were only made in multiplex families, whereas in our simplex families no deviations from the expected frequencies were observed. Possibly, this might be because of the small sample size – we have analysed only 267 simplex parental couples. We also have to take into account that this ascertainment scheme might result in a non-random distribution of parental haplotypes because there are clearly demonstrated interactions between HLA haplotypes with regard to T1D risk, making a comparison with expectations under random mating inappropriate. Nevertheless, our data indicate a different parental background of multiplex compared with simplex T1D families. These findings need to be corroborated in larger numbers, especially of simplex families.

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Fig. 1.

Association Testing in simplex and multiplex families: odds ratio (OR) and 95% confidence intervals for the DR3 and DR4 alleles using DRx (non-DR3, non-DR4) as reference.

Table 1

Analyses of the constellation of parental couples with respect to HLA-DRB1 allele/genotype frequencies in multiplex and simplex families

	Observed n (%)	Expected n (%)	p value
1815 parental couples (multiplex families)			
Both matches for			
DR3	311 (17.1)	371.4 (20.5)	0.0116
DR4	425 (23.4)	539.5 (29.7)	2×10^{-5}
DR3 or DR4	707 (39.0)	881.5 (48.6)	6×10^{-9}
DR3/4	29 (1.6)	29.4 (1.6)	0.9369
DR3/x–DR4/x	667 (36.7)	493.7 (27.2)	9×10^{-10}
267 parental couples (simplex families)			
Both matches for [†]			
DR3	51 (19.1)	57 (21.3)	0.5901
DR4	56 (21.0)	56.6 (21.2)	0.9662
DR3 or DR4	106 (39.7)	110.4 (41.3)	0.7644
DR3/4	1 (0.4)	3.2 (1.2)	0.5566
DR3/x–DR4/x [‡]	72 (27.0)	64.2 (24.0)	0.4996

[†] Both parents hold one of the cited HLA-DRB1 alleles or genotypes.

[‡] One parent possesses HLA-DRB1 genotype DR3/x and the other DR4/x (DRx = non-DR3, non-DR4).