Mode of inheritance of HLA-DRB1 shared epitope in Japanese familial rheumatoid arthritis

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Abstract

Objective. To clarify the mode of genetic contribution of the HLA-DR shared epitope (SE) to the pathogenesis of familial cases of Japanese rheumatoid arthritis (RA).

Methods. Fifty-three unrelated Japanese RA families that had more than 2 affected siblings were selected. The HLA-DR shared epitope typing was carried out by the PCR method and PCR-SSCP (single stranded DNA conformation polymorphism) method. A affected sib pair analysis was carried out using the MAPMAKER/SIB 2.0 program. The mode of inheritance was also calculated based on the sharing of genes identical by descent (IBD) between siblings in each of the 53 affected sib-pairs (propositus and the 2nd affected sib).

Results. The maximum LOD score of HLA-DR was 0.437, and the sharing of 2 IBDs, 1 IBD, and no IBDs between affected sibs were 0.330, 0.500, and 0.170, respectively. The sharing distribution of IBD was confirmed to be compatible with the dominant or additive mode since the observed gene frequency of SE was 0.255.

Conclusion. The HLA-DR shared epitope participated in the pathogenesis of familial cases of Japanese RA. The SE contributes to this pathogenesis in either the dominant or additive mode of inheritance.

Key words: HLA-DR, shared epitope, affected sibs, rheumatoid arthritis, mode of inheritance, dominant trait, additive trait.

Introduction

Following the proposal of the shared epitope hypothesis by Gregerson et al. in 1987 (1), the amino acid sequence QGLN(2)RRA(3)A on the HLA DR 1 domain was confirmed to contribute to the pathogenesis of Japanese rheumatoid arthritis (RA) (2). The QKRA(3)A sequence was shown to be a predominant RA-susceptibility sequence in many Caucasian ethnic populations and the QKRA(3)A has also been shown to contribute to RA susceptibility. The similarities of these susceptibility sequences [shared epitope (SE)] are thought to suggest a common genetic background of RA among all ethnic groups. The differences of SE among ethnic groups were possibly due to the variant distribution of the HLA-DR alleles.

The mode of the genetic trait of SE in RA has been investigated in many studies, but the results are controversial. Gremm et al. showed the dominant trait using 35 sibships (3). Strotzer et al. showed the dominant trait using multivariate RA families (4), and Torfs et al. also showed the dominant trait with the disease allele frequency of 0.254 to 0.341 (5). On the other hand, Ollier et al. (6) and Evans et al. (7) indicated the recessive mode of inheritance using a multivariate-family study and population study, respectively. Although the contribution of SE to the pathogenesis of RA is well accepted, the mode of genetic contribution of SE is still controversial. In a recent analysis on the frequency of SE using cases of familial Japanese RA with affected sibs in order to investigate the importance of SE in the pathogenesis of RA, our group found a strong contribution of SE in familial cases (8). In view of the apparently strong contribution of genetic factors in these cases, we conducted this brief study to investigate the mode of genetic contribution of HLA-DR. The LOD score was also examined by affected sib pair analysis using the MAPMAKER/SIB 2.0 program.

 Patients and methods

Patients

Unrelated Japanese RA families that had more than two affected sibs were selected. All propositi and second affected sibs were the same as the members reported in our previous paper (8). In brief, 53 propositi from different families (45 women and 8 men, aged 58.7 ± 9.5 [mean ± SD]) were diagnosed according to the criteria of the American Rheumatism Association. The positivity of rheumatoid factor (RAF) was 86.8%. The 53 affected sibs (2nd affected sib) of the propositi (40 women and 13 men) were aged 57.1 ± 8.9. In 5 families, the samples consisted of either of the parents. In 2 families in this group, the samples consisted of
additional sibs, excluding 2 affected sibs, and in 1 family the samples consisted of children. Among the 48 families without parents' samples, the samples in 17 families consisted of additional sibs, excluding 2 affected sibs, and the samples in 5 families consisted of children. The samples in 28 families consisted of only 2 affected sibs. Genomic DNA from white blood cells was obtained from all available family members (8).

**HLA-DR shared epitope (SE) typing**

First, HLA-DR typing was performed by the polymerase chain reaction (PCR) method using the DR group specific primers described previously (8). All propositi and second affected sibs and control subjects had already been genotyped previously and phenotype frequencies and genotype frequencies were shown in our previous report (8). Other familial members available were genotyped to confirm the genotypes of the propositi and second affected sibs.

**Genetic analysis**

Affected sib pair analysis was carried out using the MAPMAKER/SIB 2.0 program (Whitehead Institute for Biomedical Research, http://www.genome.wi.mit.edu) (9). The LOD score was also calculated by this program. HLA-DR was analyzed in the "single-point" mode. The mode of inheritance was calculated according to formulas of Thomson and Bodmer (10) and Thomson (11).

**Statistics**

The $^2$ test was used for comparing the observed number with the expected number in the 2 x 3 table. Significance (p value) was calculated using Excel 97 for Windows. Genotype frequencies (GF) were occasionally shown and compared by the Fisher’s exact test.

**Results**

Genotype frequencies of HLA DRB1*0405 and SE were 0.175 and 0.255, respectively, in normal Japanese (8). The genotype frequencies of DRB1*0405 and SE in propositi were significantly higher than those in normal controls (p = 0.039, odds ratio (OR) = 1.86 (95% confidence interval (CI): 1.07 - 3.25), and p = 0.0019, OR = 2.24 (CI:1.36 - 3.69), respectively, as was shown in our previous report (10).

The linkage analysis between RA and HLA DRB1 by the affected sib pair method using the MAPMAKER/SIB 2.0 program showed a maximum LOD score of 0.437 (single-point analysis).

The sharing of IBD observed in Japanese familial RA was 0.330: 0.500: 0.340 0.338 0.485 0.170 0.977 at the gene frequency of 0.255 was 0.845 (Table I). On the other hand, under the assumption of recessive trait, the sharing pattern showed the highest p value of 0.982 at the gene frequency of 0.724. When the gene frequency of SE was 0.255, the expected IBD sharing distribution was significantly different from the observed IBD sharing distribution ($p = 3.92 \times 10^{-4}$) (Table I). Considering that the gene frequency of SE was 0.255, dominant or additive trait was compatible to the observed IBD sharing.

**Discussion**

In this study, DRB1 was shown to have either a dominant or additive trait under the assumptions that the gene frequency of SE was 0.255, and the recessive trait was denied, although this result was preliminary using limited number of affected-sib RA families. If the recessive trait was assumed, the gene frequency would have to be more than 0.450 (p=0.05 at the gene frequency of 0.347. The p value at the gene frequency of 0.255 was 0.845 (Table I). On the other hand, under the assumption of recessive trait, the sharing pattern showed the highest p value of 0.982 at the gene frequency of 0.724. When the gene frequency of SE was 0.255, the expected IBD sharing distribution was significantly different from the observed IBD sharing distribution ($p = 3.92 \times 10^{-4}$) (Table I). Considering that the gene frequency of SE was 0.255, dominant or additive trait was compatible to the observed IBD sharing.

<table>
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<tr>
<th>Mode</th>
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<th>Shared IBD Z1</th>
<th>Z0</th>
<th>p</th>
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</table>

Gene frequency of SE and HLA-DR*0405 were 0.255 and 0.175 in Japanese normal, respectively. The observed IBD sharing was calculated using MAPMAKER/SIB 2.0 (9) and the expected IBD sharing was calculated according to formulas of Thomson and Bodmer (10) and Thomson (11).
Genetic mode of shared epitope in familial RA / F. Takeuchi et al.

0.450, and p = 0.257 at the gene frequency of 1.000) to explain the observed sharing of DRB1 in RA. These observations strongly suggest the dominant or additive contribution of SE to Japanese familial RA. It is not clear, however, whether the trait is dominant or additive, because it is difficult, in general, to calculate the difference of penetrance between homozygote and heterozygote in diseases of dominant trait. Moreover, the ratio of sharing of IBD calculated under the consumption of dominant and that under the consumption of additive mode of inheritance are nearly equal (10, 11). These two modes could not be separated clearly in our study (12).

Dominant and additive contributions of HLA to RA have been shown in several reports (3-5, 12-14). The HLA DR alleles were shared between affected sibs at a distribution of 0.330: 0.500: 0.170 (Z2:Z1:Z0) in this study. Strotzer (4) summed up several reports and showed the distribution of the haplotype sharing of HLA DR alleles as 0.31:0.50:0.19 (Z2:Z1:Z0). Torfs also summed up and showed the distribution as 0.33: 0.51: 0.16 (5). Both of these distributions were almost the same as that in this study, and both reports showed dominant trait. Wallin et al. (12) reported a dominant or additive mode of inheritance of SE in RA. Yamashita et al. (13) reported a tight linkage between the putative RA susceptibility gene and HLA-DR under an autosomal dominant mode of inheritance using familial RA. Gao et al. also show HLA-DR as a dominant risk factor (14). Our results using the method of HLADRBI genotyping were compatible with these reports. Despite our results and previous reports indicating dominant and additive contribution of HLA DR, Ollier et al. (6) reported that analysis of Hardy-Weinberg equilibrium for DR using patients from the London Hospital did not support dominant or additive trait. Evans et al. (7) showed recessive mode in Caucasian patients. Rigby et al. also rejected an additive (dominant) trait using almost the same method (15).

The reason for the discrepancies in the results remain unclear. There is a possibility that the genetic factors of RA are different among ethnics. In insulin-dependent diabetes mellitus, HLA-DQ was reported to be a susceptibility gene in Caucasians, while HLA-DR was suggested to be of importance in Japanese (16). Some environmental factors may affect the penetrance. Gender or other genes may affect on autosomal genes like HLA-DR (17). In the case, HLA-DR would not be major susceptibility gene. It is suggested, at least in Japanese familial cases in whom genetic factors are presumed to contribute strongly, that HLA DR dominantly or additively contributes to the pathogenesis of RA.

The maximam LOD score of the HLA-DR gene in this study was 0.437, although the result was preliminary using limited number of affected-sib RA families. The LOD score was a relatively low score considering the significant association between SE and RA. Go et al. (18) also reported a low LOD score of the HLA-DR gene in spite of a significant association between RA and DR. Shiozawa et al. (19) reported a low LOD score using Japanese affected sibs. The reason for the relatively low LOD score may be due to the relatively small number of samples. The heterogeneity would affect it. Go et al. postulated that this data might suggest a contribution of non-major histocompatibility complex genes to the development of RA (18).

Our results showed that SE dominantly or additively participates in the pathogenesis of Japanese familial RA, but this participation was neither unique nor indispensable. We know this because the relatively low odds ratio of SE has been confirmed in various studies, and it is also well known that substantial numbers of RA patients do not have SE, and that patients without SE often show typical features of RA. These observations demonstrate the possible contribution of not only SE but also other genetic factors in the pathogenesis of RA. Cornelis et al. reported that HLA was one of the important factors for pathogenesis of RA, and they also showed the possible contribution of other gene(s) located at 3q13 (20). Jawaheer et al. (21), Strotzer et al. (4) and Go et al. (18) also suggested that other genes might take part in the pathogenesis of RA. In the near future, all genetic factors of RA will be defined and the relationship of SE with those factors will be clarified.

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References


