Genetics of Microscopic Polyangiitis in the Japanese Population

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The epidemiology of ANCA-associated vasculitis is substantially different between Caucasians and Japanese, which may be related to differences in genetic backgrounds. In this review, I discussed our findings on the genetics of microscopic polyangiitis (MPA) in Japanese. Analysis of HLA genes revealed a significant increase in the HLA-DRB1*09:01-DQB1*03:03 haplotype MPA. This is one of the most frequent haplotypes in Japanese, but is nearly absent in Caucasians, and has been shown to be associated with multiple autoimmune diseases.

Analysis of KIR genes revealed significant decreases in the carrier frequency of an activating receptor KIR2DS3 in MPA. When KIRs were analyzed in combination with HLA ligands, the proportion of individuals carrying KIR3DL1 and HLA-Bw4 but not KIR3DS1, the most inhibitory of all KIR3DS1/3DL1/HLA-B combinations, was significantly increased in MPA. These results suggested that decreased activation of NK and/or T cells may cause a predisposition to MPA.

LILRA2 is an activating receptor involved in granulocyte and macrophage activation. LILRA2 SNP rs2241524 G >A, which disrupts the intron 6 splice acceptor site, was significantly associated with MPA. The risk allele produces an LILRA2 isoform lacking three amino acids in the linker region.

These findings, when confirmed by larger-scale studies, will shed light on the molecular mechanisms of MPA. (*English Translation of J Jpn Coll Angiol 2009; 49: 31-37.)

Keywords: microscopic polyangiitis, genetics, HLA, KIR, LILRA2

INTRODUCTION

Among anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitides, granulomatosis with polyangiitis (GPA) is frequently observed in Western countries, particularly in northern Europe, whereas microscopic polyangiitis (MPA) is frequently observed in Japan. It is speculated that such differences among populations are associated with certain genetic and environmental factors. A possible environmental factor is microbial infection. Studies on human ANCA-associated vasculitis have suggested the involvement of Staphylococcus aureus or cytomegalovirus (CMV) infection. Another article of this special feature introduces studies suggesting a microbial contribution in model animals with vasculitis. At present, it may be appropriate to consider that individual differences in responses to environmental factors such as microorganisms are involved in the development of ANCA-associated vasculitis.

Disorders such as ANCA-associated vasculitides have few clues to their pathogenesis, as such, the identification of disease susceptibility genes is expected to provide clues to clarify pathogenesis and essential pathology and develop treatment methods. However, since their incidences are low, it has been difficult to collect disorder-prone families necessary for genome-wide linkage analysis.
and samples from a thousand or more patients necessary for reliable genome-wide association studies. At present, candidate gene approaches based on the results of pathological analysis in humans and results from animal models have been mainly performed.

Immune system genes are major factors for biological responses to environments and may be promising candidate genes since marked functional diversity has been acquired due to microorganisms as selection pressure in human populations. Concerning the genetic background of ANCA-associated vasculitis in Japanese, we as members of “The Research Committee of Intractable Vasculitis Syndrome” of the Ministry of Health, Labor, and Welfare of Japan evaluated immune system genes as candidate genes. Although this research is a multi-institutional joint study, due to the low incidences of disorders, the number of samples that could be analyzed was only 50 for MPA, 8 for GPA, and 8 for eosinophilic granulomatosis with polyangitis (EGPA). Therefore, statistical analysis was mainly focused on MPA. Thus far, relatively small number of studies have been published on genetic predispositions for ANCA-associated vasculitis, most of which came from Western countries. In addition, although ANCA-associated vasculitis is mostly observed in Japan and Western countries, its epidemiology considerably differs between the two regions. Therefore, in this paper, we do not provide general remarks about ANCA-associated vasculitis, but introduce the results from our research committee.

**Human Leukocyte Antigen (HLA) Genes**

HLA molecules present antigens to T cells and show extensive polymorphism directly affecting immune responses. Since HLA genes are the most established disease susceptibility genes in many immunological disorders and infections, we initially analyzed them as candidate genes.

Studies on the association between HLA and ANCA-associated vasculitis have shown associations between GPA and HLA-DR4 as well as DR1 in Europe. In Japan, before our study, studies in a few cases have shown an increase in HLA-DR9 in GPA and MPO-ANCA-associated glomerulonephritis. We performed association studies between ANCA-associated vasculitis and the HLA-DRB1 genotype and observed HLA-DRB1*09:01 in 29.1% of the healthy control group but 50% of the MPA group, showing a significant increase in the latter (P = 0.037; odds ratio [OR], 2.44). In addition, HLA-DQB1, DPB1, B, and C were evaluated and a significant association between MPA and DQB1*03:03 (OR, 2.35) was observed. Due to the presence of marked linkage disequilibrium between HLA-DRB1*09:01 and DQB1*03:03, it was impossible to determine which of them is the primary susceptibility gene. On the other hand, MPA was not clearly associated with HLA-B, C, or DPB1. Thus, in the Japanese population, the HLA-DRB1*09:01-DQB1*03:03 haplotype was found to be a significant genetic factor for MPA.

The HLA-DRB1*09:01-DQB1*03:03 haplotype has two unique characteristics. One is that this haplotype is frequently observed in Asian populations such as Japanese but is rarely present in European or African populations. The other is the association between this haplotype and various autoimmune diseases such as type I diabetes mellitus, juvenile myasthenia gravis, anti-phospholipid antibody production, and anti-CCP antibody-negative rheumatoid arthritis in Japanese. This is like a mirror image of the HLA-DRB1*03:01 haplotype, which is frequently observed in populations in northern Europe, but is absent in Japanese and is associated with various autoimmune diseases.

The function of HLA-DR and DQ is antigen presentation to T cells. Therefore, one possible molecular mechanism that can explain the association between the HLA-DRB1*09:01-DQB1*03:03 haplotype and MPA is specific presentation of a certain pathogenic antigen peptide by one of the following molecules: HLA-DR9 molecule, which is a heterodimer of the HLA-DRB1*09:01 allele product and HLA-DRA1 allele product, the HLA-DQ9 molecule, which is a heterodimer of HLA-DQB1*03:03 and the DQA1*03 allele products encoded by this haplotype, and the HLA-DR53 molecule, which is a heterodimer of HLA-DRB4*01 and DRA1 products (Fig. 1).

However, it may be difficult to explain the association between HLA genes and diverse disorders based on binding of a specific antigen peptide. HLA genes are encoded on human 6p21.3. On this region, genes other than HLA genes (TNFα, C4, and TAP genes) are also encoded and linkage with HLA is observed. Considering that this haplotype is associated with various autoimmune diseases, it is more likely that polymorphism of immune system genes (other than HLA genes), which are encoded on this haplotype, rather than presentation of a specific antigen peptide is causally associated with MPA (Fig. 1). The two possibilities should be taken into consideration for clarification of the molecular mechanism.

In addition, as described above, the prevalence of GPA...
Genetics of MPA in the Japanese Population

or MPA markedly differs among populations. Further studies in various populations are necessary to determine whether these differences are caused by differences in \textit{HLA} among populations.

\textbf{Killer Cell Immunoglobulin-Like Receptor (KIR) Genes}

Human NK cells express many activating and inhibitory receptors. Inhibitory receptors recognize self \textit{HLA}-class I molecules expressed in most cells and transmit inhibitory signals, preventing the self-cytotoxicity of NK cells. In situations such as the presence of tumor cells and virus-infected cells in which self \textit{HLA}-class I expression is inhibited, inhibitory signals decrease, resulting in cytotoxicity against such cells (missing self-theory).\textsuperscript{14} KIR, belonging to the immunoglobulin superfamily, is expressed on NK cells and some subpopulations of T cells and there are both activating and inhibitory types.

KIR is encoded on 14 genetic loci adjacent to leukocyte Ig-like receptor (LILR) genes in the leukocyte receptor complex (LRC) located on human chromosome 19q13.4. KIR molecules are termed according to the number of the extracellular Ig domain and the length of the intracellular domain. For example, KIR3DL1 has three extracellular Ig domains and a long intracellular domain, and KIR2DS1 has two extracellular Ig domains and a short intracellular domain. In general, the long domain (DL) group includes inhibitory receptors with immunoreceptor tyrosine-based inhibitory motifs (ITIM) in the intracellular domain while the short domain (DS) group has a positively charged amino acid (Lys) in the membrane spanning domain and is coupled to immunoreceptor tyrosine-based activation motif (ITAM)-containing DAP12 as an adaptor molecule, transmitting activating signals. However, only KIR2DL4 in the DL group has positively-charged Arg in the membrane spanning domain and is coupled to immunoreceptor tyrosine-based activation motif (ITAM)-containing DAP12 as an adaptor molecule, transmitting activating signals.\textsuperscript{15}

KIR, unlike T cell receptors, recognizes subgroups of \textit{HLA}-class I, classified according to the amino acid sequence motif (Table 1).

\textit{KIR} genes show not only base sequence polymorphism but also extensive polymorphism due to the presence or absence of loci themselves. As a result, the number of \textit{KIR} genes in the genome markedly varies (7–14) among individuals. For details of this variation, refer to other reviews.\textsuperscript{16–18}

\textit{KIR} and \textit{HLA} are encoded on difference chromosomes and, therefore, are inherited independently. Combinations of \textit{KIR} and \textit{HLA} as a ligand, both of which are
extensively polymorphic, cause genetic individual differences in signal transmission mediated by HLA-KIR. With this background, an association between KIR/HLA combinations and virus infection particularly that due to HIV, HCV, and human papillomavirus, or autoimmune diseases such as rheumatoid arthritis, psoriasis vulgaris, psoriatic arthritis, type I diabetes mellitus, and scleroderma has been reported. The results of previous studies can be summarized as follows. The risk of developing autoimmune diseases tends to increase when activating signals assumed by HLA-KIR combinations are intense while the risk of virus infection tends to increase when inhibitory signals are intense. However, in uterine cervix cancer associated with virus infection, inhibitory combinations decrease the risk of developing this disease, which is considered to be because persistent inflammatory reactions in the host to virus infection are associated with carcinogenesis.

Each NK cell in individuals expresses their KIR family gene clusters in a random fashion to some extent. Therefore, in the same individuals, there are NK cells expressing and those not expressing a certain type of KIR. In a child with repeated CMV infection, expression of an inhibitory receptor KIR2DL1 as in all NK cells was reported. This finding supports the above results that resistance to virus infection decreases in individuals assumed to have intense inhibitory signals mediated by KIR.

In view of this background, we analyzed the presence or absence of KIR genes and KIR/HLA ligand combinations in Japanese subjects with MPA.

Initially, the presence or absence of 14 KIR loci was determined in 443 Japanese patients with MPA and 239 healthy controls. An activating receptor KIR2DS3 was positive in 16.7% of healthy controls but 4.7% of patients with MPA, showing a significant decrease in the latter (P = 0.038).

Subsequently, KIR/HLA ligand combinations were evaluated. No significant differences were observed in the frequency of HLA-B or HLA-C alone between MPA and control groups. However, the frequency of the combination of HLA-Bw4+, KIR3DL1+, and KIR3DS1-, which is considered to be the most inhibitory among the combinations between KIR3DL1/3DS1 and HLA-Bw4, was significantly higher (P = 0.014) in the MPA group (46.5%) than that in the control group (27.0%) with an odds ratio of 2.35 (Fig. 2).

Furthermore, HLA-C was also incorporated as a ligand into this analysis. Combinations that are considered to be more inhibitory showed a more marked increase in the risk of developing MPA. These results suggest an association between inhibition of NK cells and T cells mediated by KIR and the risk of developing MPA.

These results together with the above previous reports concerning autoimmune diseases and virus infection suggest that combinations associated with decreased resistance to virus rather than autoimmune diseases are susceptible to MPA. As described at the beginning, some studies have suggested the causal involvement of Staphylococcus aureus or CMV in ANCA-associated vasculitides. In addition, MPA develops at advanced age and is often complicated by severe intractable infection such as CMV pneumonia during its course. These findings may allow the proposal of the following hypothesis: Combination of an age-related decrease in resistance and a genetic decrease in resistance to infection due to the KIR genotype causes persistence and aggravation of virus infection, and this condition, together with multiple genetic predispositions and environmental factors, eventually leads to ANCA production and results in the development of vasculitis.

### Table 1 KIRs and their ligands

<table>
<thead>
<tr>
<th>KIR</th>
<th>Ligand</th>
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<tbody>
<tr>
<td>KIR2DL1</td>
<td>HLA-Cgroup2</td>
</tr>
<tr>
<td>KIR2DL2/3</td>
<td>HLA-Cgroup1</td>
</tr>
<tr>
<td>KIR2DL4</td>
<td>HLA-G</td>
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<tr>
<td>KIR2DL5</td>
<td></td>
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<tr>
<td>KIR3DL1</td>
<td>HLA-Bw4</td>
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<tr>
<td>KIR3DL2</td>
<td>HLA-A3, A11</td>
</tr>
<tr>
<td>KIR3DL3</td>
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<tr>
<td>KIR2DS1</td>
<td>HLA-Cgroup2</td>
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<tr>
<td>KIR2DS2</td>
<td>(HLA-Cgroup1)</td>
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<tr>
<td>KIR2DS3</td>
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<td>KIR2DS4</td>
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<td>KIR2DS5</td>
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<tr>
<td>KIR3DS1</td>
<td>(HLA-Bw4)</td>
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</table>

HLA-Cgroup2 consists of HLA-Cw alleles possessing Asn77 and Lys80, such as Cw2, 4, 5, and 6. HLA-Cgroup1 includes HLA-Cw1, 3, 7, and 8, which contain Ser77 and Asn80. HLA-Bw4 is defined by position 77-83 amino acids and includes HLA-B13, B27, B44, B51, B52, and A24. A weak interaction has been postulated between KIR2DS2 and HLA-Cgroup1 and between KIR3DS1 and HLA-Bw4, which has not been conclusively established.
LILRA2

For **LILR** (also called **ILT** or **CD85**) genes adjacent to **KIR** genes in LRC, there are 13 genetic loci including those for 2 pseudogenes. LILR molecules are classified into activating (**LILRA1, -A2, -A4, -A5, and -A6**), inhibitory (**LILRB1, -B2, -B3, -B4, and -B5**), and soluble (**LILRA3, -A5s**) types. Although the cell type distribution differs among LILR types, these receptors are generally expressed in the leukocyte line, and some of them use HLA-class I or UL18 of CMV as a ligand.

**LILRA2** is involved in the activation of eosinophils, basophils, and macrophages. This type has also been reported to exert an inhibitory action on immune responses by shifting IL-12 production to IL-10 production in monocytes and inhibiting TLR signals.

Regarding **LILR** genes as candidate genes, we conducted an association study on autoimmune rheumatic diseases, and during this process, found an association between SNP rs2241524 replacing the splice acceptor site at the intron 6-exon 7 junction of **LILRA2** and systemic lupus erythematosus (SLE) as well as MPA. The A/A genotype was observed in 7.0% of the healthy control group but 12.1% of the SLE group (\(P = 0.041; \text{OR} = 3.55\)) and 16.0% in the MPA group (\(P = 0.049; \text{OR} = 2.52\)), showing significant increases.

Since this SNP was expected to change the splicing site, the cDNA sequence was evaluated for each genotype. When an A allele is present, the cryptic splice site 9 nucleotides downstream of the original splice acceptor site is used, and, therefore, translation to protein with deletion of 3 amino acids corresponding to positions 419–421 in the amino acid sequence was considered.

**Fig. 2** HLA-Bw4, KIR3DL1, and KIR3DS1 combinations in Japanese MPA and healthy controls.

Each individual can be grouped into 6 groups according to the presence/absence of KIR3DL1, KIR3DS1, and HLA-Bw4, the ligand of KIR3DL1 (and possibly of KIR3DS1). KIR3DL1 contains ITIM and transmits inhibitory signals, while KIR3DS1 associates with DAP12, which contains ITAM and transmits activation signals. The frequencies of each group in Japanese patients with MPA and controls are shown on the right. The most inhibitory combination, **HLA-Bw4+, KIR3DL1+, KIR3DS1-** (top), was significantly increased in MPA. Because the ligand of KIR3DS1 has not been identified, here we assumed an unknown ligand expressed in all individuals. It should be noted that there is a possibility that KIR3DS1 weakly interacts with HLA-Bw4, as described in **Table 1**. However, this does not significantly affect the above interpretation.

OR: odds ratio; CI: confidence interval
In individuals with the A/A genotype, 100% of mRNAs showed this Δ419–421 isoform. Positions 419–421 correspond to the linker region that is present between the extracellular Ig-like domain and membrane spanning domain. Studies using monoclonal antibodies have confirmed that this isoform is also expressed on the monocyte surface. Further studies are necessary on changes in LILRA2 signals due to this deletion of 3 amino acids.

**CONCLUSIONS**

Concerning the genetic predispositions for MPA, we have mainly evaluated multigene families showing extensive functional polymorphisms (HLA, KIR, and LILR) among gene clusters in the immune system. MPA is a rare disease and only an extremely few cases have been evaluated in our studies as genetic studies. Therefore, further studies are necessary on the reproducibility of results. However, our and other previous studies have shown an association between each of these genes and other autoimmune diseases or infections, which supports the possibility that these polymorphisms have functional significance.

In the future, the associations found in this study and molecular mechanisms should be further evaluated for the clarification of pathogenesis and drug discovery. If hundreds of samples can be collected from Japanese with ANCA-associated vasculitis, a large-scale susceptibility gene analysis becomes possible by genome wide association studies. Joint research systems at the national level and in Asian populations are necessary in the future.

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