Analysis of Single Nucleotide Polymorphisms of the Aryl Hydrocarbon Receptor in Japanese Psoriasis Patients

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Abstract  The aryl hydrocarbon receptor (AHR) is a dioxin receptor that activates the differentiation of T helper 17 (Th17) cells and regulatory T (Treg) cells to produce interleukin-22 (IL-22). In whole exome sequence analysis, we found two single nucleotide polymorphisms (SNPs) in AHR, rs2074113 and rs2066853. In addition, we investigated the correlation between these AHR SNPs and susceptibility to psoriasis in 185 patients with psoriasis vulgaris (PV) and 145 healthy controls by using TaqMan® SNP Genotyping assays. For rs2074113, in both groups the frequency of the G allele was 0.59 and that of the T allele was 0.41. For rs2066853, in both groups the frequency of the A allele was 0.42 and that of the G allele was 0.58. Thus both allele frequencies in both groups were not significantly different and therefore the frequency of AHR SNPs and susceptibility to PV were not correlated. However, in a comparison of HLA-C*06:02-positive and -negative patients, rs2066853 AA homozygotes were significantly decreased in HLA-C*06:02-positive PV patients compared to HLA-C*06:02-negative PV patients after Bonferroni correction. These observations suggested that AHR rs2066853 may be a protective gene for psoriasis in the Japanese population.

Key words: aryl hydrocarbon receptor, psoriasis vulgaris, single nucleotide polymorphism, disease susceptibility to psoriasis

Introduction

The aryl hydrocarbon receptor (AHR) is a ligand-dependent transcription factor whose gene is located on human chromosome 7. The AHR is best known to mediate the effects of environmental toxins (e.g., dioxin). Additionally, the AHR has been previously shown to be involved in T cell differentiation and function and AHR is a key factor in the production of interleukin-22 (IL-22) by Th17 cells and Th22 cells.

Psoriasis is a common, chronic, T-cell-mediated inflammatory disease of the skin. The most common form of psoriasis, psoriasis vulgaris (PV), is characterized by varying numbers of red, raised, scaly, skin patches that can be present on any surface of the body.

The genetics of psoriasis are complex, and this disease is highly heritable. However, it is clear that environmental effects are also responsible for disease susceptibility to psoriasis. Ten genome-wide linkage scans have resulted in strong evidence of a susceptibility locus (PSORSI) in the major histocompatibility complex (MHC) on chromosome 6p21 but have not yielded consistent evidence regarding the involvement of other chromosomal regions. Linkage and association in the MHC (6p21) are thought to be due to the human leukocyte antigen (HLA)-C. In particular, psoriasis-susceptibility effects are thought to be caused by the HLA-C*06:02 allele, although other candidate genes in the region may also contribute to disease disposition.
In this study, we analyzed the association between PV and AHR single nucleotide polymorphism (SNP) frequency in Japanese PV patients with or without the HLA-C*06:02 gene.

Patients, materials and methods

Study samples
This study involved 185 patients with PV and 145 healthy controls. Patient age ranged from 12 to 91 years, with a median age of 53 years. One hundred and forty-eight patients (80%) were male and 37 (20%) were female. The age of healthy controls ranged from 22 to 54 years, with a median age of 28 years. Eighty controls (55%) were male and 65 (45%) were female. The age and sex ratio of patient and control groups were not in concordance. All patients and controls provided their written informed consent prior to participation according to the protocols approved by the Ethics Review Committee of Gene Analysis Research, Yamaguchi University School of Medicine and University Hospital. DNA was extracted using the QIAamp DNA Blood Maxi Kit (Qiagen, Hilden, Germany).

Whole-exome sequencing
Four samples were prepared for whole-exome sequencing (WES): one from a patient with PV (a 68 year-old man); two from patients with generalized pustular psoriasis (GPP) (a 50 year-old woman and a 51 year-old man), and one from a healthy control (a 41 year-old woman).

Briefly, 3 μg of genomic DNA was sheared into 150-200 base pair-long fragments using the Covaris DNA Shearing System. To capture exonic DNA, the SureSelectXT Human All Exon v5 capture library (Agilent) was used. The sequence library was constructed with the SureSelectXT Target Enrichment System for Illumina Multiplexed Sequencing according to the manufacturer’s instructions. DNA sequencing of 100-bp paired-end reads was performed using the Illumina Hiseq 2000 sequencer.

Exome Data Analysis
The WES data were analyzed by SNP analysis and insertion and deletion (indel) calling. Sequencing data were processed using Illumina Real Time Analysis (RTA). Briefly, the WES reads were aligned to the Human reference genome (GRCh37/hg19). The alignment was executed with the Burrows-Wheeler Aligner (BWA) and variant calling was performed with the Genome Analysis Toolkit (GATK). GATK parameters included base quality score recalibration and duplicate removal. The region information of variants was annotated using SnpEff.

SNP Selection for Follow-Up Genotyping
In patients with PV, variation in AHR was observed in two SNPs, rs2074113 (c.908+33G>T) and rs2066853 (c.1661G>A) (Table 1 and Fig. 1), which were then selected for follow-up genotyping.

TaqMan® SNP Genotyping

Table 1  Results of whole-exome sequencing in patients with PV and GPP and in healthy controls. Two AHR SNPs were found.

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>ID</th>
<th>Rank</th>
<th>ALT</th>
<th>SNP</th>
<th>DNA changes</th>
<th>PV</th>
<th>GPP ①</th>
<th>GPP ②</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHR</td>
<td>rs2074113</td>
<td>Intron 7</td>
<td>G</td>
<td>T</td>
<td>c.908+33G&gt;T</td>
<td>G/G</td>
<td>G/T</td>
<td>G/G</td>
<td>T/T</td>
</tr>
<tr>
<td>AHR</td>
<td>rs2066853</td>
<td>Exon 10</td>
<td>G</td>
<td>A</td>
<td>c.1661G&gt;A</td>
<td>G/G</td>
<td>A/G</td>
<td>G/G</td>
<td>A/A</td>
</tr>
</tbody>
</table>

*GPP①: woman, 50 years old
*GPP②: man, 51 years old
PV = psoriasis vulgaris
GPP = generalized pustular psoriasis
The SNPs AHR rs2074113 and rs2066853 were genotyped using TaqMan® SNP genotyping assays (C_16163703_10 and C_11170747_20, respectively) with probes and primers designed and synthesized by the supplier (Applied Biosystems). The reaction was performed on a Steponeplus™ Real Time PCR System (Applied Biosystems) according to the manufacturer’s instructions, in a total volume of 10 μl containing 0.25 μl of TaqMan® SNP Genotyping Assays 40 × , 5 μl of TaqMan2 × Genotyping Master Mix (Applied Biosystems) and 10 ng of DNA sample diluted in 3.75 μl of DNase-free water. The following are the conditions used for the polymerase chain reaction: 95 ℃ for 10 minutes and 40 cycles of 95 ℃ for 15 sec and 60 ℃ for 1 minute.

DNA typing of HLA-C genes

HLA-C alleles were identified by PCR with sequence-specific primers (PCR-SSP) as described previously. Briefly, 10 μg of genomic DNA was used to amplify each HLA-C gene by PCR with specifically designed primers. Alleles were assigned by the reaction patterns of the sequence-specific oligonucleotide probes (One Lambda, Inc., Canoga Park, CA, USA).

Statistics

The differences in allele frequencies between the PV patients and the healthy controls were assessed by Fisher’s exact test. The Hardy-Weinberg equilibrium was tested in the first step of statistical analysis by the χ² test. The Bonferroni correction was applied to address the problem of multiple comparisons.

Results

Genotype and allele frequencies of AHR rs2074113

Analysis of the frequencies of the genotypes of the AHR SNP rs2074113 (G/G, G/T, or T/T) indicated that, in the PV group (n=185), the frequency of G/G was 38% (70/185), of G/T was 42% (78/185), and of T/T was 20% (37/185). In the healthy control group (n=145), the frequency of G/G was 33% (48/145), of G/T was 51% (74/145), and of T/T was 16% (23/145) (Table 2). There were no significant differences between the two groups in terms of the frequency of any of the genotypes. The populations of genotypes agreed with the Hardy-Weinberg equilibrium (χ² = 0.39). Analysis of the frequencies of these G and T alleles indicated that the frequency of the G allele was 0.59, and that of the T allele was 0.41, for both the PV and the healthy control group (Table 2). Thus there were no significant differences in the frequencies of the G or T alleles between the two groups.

Genotype and allele frequencies of AHR rs2066853

Analysis of the frequencies of the genotypes of the AHR SNP rs2066853 (G/G,
G/A, or A/A) indicated that, in the PV group (n=185), the frequency of G/G was 35% (66/185), of G/A was 45% (83/185) and of A/A was 20% (36/185). In the healthy control group (n=145), the frequency of G/G was 31% (46/145), of G/A was 52% (75/145), and of A/A was 17% (24/145) (Table 2). There were no significant differences between the two groups in terms of the frequency of any of the genotypes. The populations of genotypes agreed with the Hardy-Weinberg equilibrium ($\chi^2 = 0.54$). Analysis of the frequency of these G and A alleles indicated that the frequency of the G allele was 0.58 and that of the A allele was 0.42 for both the PV and the healthy control group (Table 2). There were no significant differences in the frequencies of the G or A alleles between the two groups.

### HLA-C*06:02 frequencies

The frequency of HLA-C*06:02 genes in the PV group (n=185), the frequency of G/G was 35% (66/185), of G/A was 45% (83/185) and of A/A was 20% (36/185). In the healthy control group (n=145), the frequency of G/G was 31% (46/145), of G/A was 52% (75/145), and of A/A was 17% (24/145) (Table 2). There were no significant differences between the two groups in terms of the frequency of any of the genotypes. The populations of genotypes agreed with the Hardy-Weinberg equilibrium ($\chi^2 = 0.54$). Analysis of the frequency of these G and A alleles indicated that the frequency of the G allele was 0.58 and that of the A allele was 0.42 for both the PV and the healthy control group (Table 2). There were no significant differences in the frequencies of the G or A alleles between the two groups.

### Frequency distribution of AHR rs2074113 genotypes and alleles among HLA-C*06:02-positive PV and HLA-C*06:02-negative PV

Analysis of the frequencies of the genotypes of AHR rs2074113 in the HLA-C*06:02-positive PV group (n=19) indicated that the frequency of G/G was 53% (10/19), of G/T was 42% (8/19), and of T/T was 5% (1/19). In the HLA-C*06:02-negative PV group (n=166), the frequency of G/G was 33% (55/166), of G/A was 45% (75/166), and of A/A was 22% (36/166) (Table 4). There were no significant differences in the frequency of these genotypes and alleles between HLA-C*06:02-positive and HLA-C*06:02-negative PV (Table 4).

### Frequency distribution of AHR rs2066853 genotypes and alleles among HLA-C*06:02-positive PV and HLA-C*06:02-negative PV

Analysis of the frequencies of the genotypes of AHR rs2066853 in the HLA-C*06:02 positive PV group (n=19) indicated that the frequency of G/G was 58% (11/19), of G/A was 42% (8/19), and of A/A was 0% (0/19). In the HLA-C*06:02-negative PV group (n=166), the frequency of G/G was 36% (60/166), of G/T was 42% (70/166), and of T/T was 22% (36/166) (Table 4). The frequency of A/A in HLA-C*06:02-positive PV patients was significantly higher (p = 1.09 × 10^{-5}) than in the healthy controls.
significantly decreased compared to that in HLA-C*06:02-negative PV (A/A vs. G/G: \( p = 0.0073 \)). \( P < 0.0167 \) was considered statistically significant due to Bonferroni corrections for multiple comparisons (Bonferroni corrected \( p \)-value: 0.05/3 = 0.0167). Analysis of the frequency of these G and A alleles in the HLA-C*06:02 positive PV group and HLA-C*06:02 negative PV group, indicated that the frequency of the A allele was significantly decreased in HLA-C*06:02-positive PV (\( p = 0.0055 \)) (Table 4).

**Discussion**

The present study investigated the association of AHR SNPs with PV in a Japanese population. Based on the result of whole exome sequencing, we studied two SNPs in the AHR gene and investigated the relationship between AHR genetic polymorphisms and the onset of PV. No evident associations were noted, and it was not possible to conclude that these AHR polymorphisms serve as genetic factors that can determine susceptibility to PV. HLA-C*06:02 is generally regarded as the top risk allele for PV susceptibility,^{13} and the frequency of the HLA-C*06:02 gene was significantly increased in the psoriasis group compared with that in the controls in this study (Table 3). In spite of this finding, AHR rs2066853 AA homozygous was not more prevalent and was in fact significantly decreased in HLA-C*06:02 positive PV patients than in HLA-C*06:02 negative PV patients. Thus, AHR rs2066853 AA homozygous may play important roles in the protection of psoriasis patients from the development of PV.

An important role of AHR is to induce the differentiation of Th17 and Th22 cells^{6,7} to produce interleukin-22 (IL-22)^{6,8} and this process has provided targets for recent biological drug development for the treatment of psoriasis. Th22 cells are a recently described subset of IL-22-producing cells that do not express IL-17A or IFN-\( \gamma \)^{15-18} In the skin, IL-22 mediates keratinocyte proliferation and epidermal hyperplasia and is thought to play a central role in psoriasis. The IL-17A antibody, Secukinumab, has already been approved for moderate-to-severe plaque psoriasis
management. Since neutralization of IL-22 has been reported to prevent the development of psoriasis-like skin lesions in mice, it is considered that the IL-22 antibody might also prove to be a new therapy for the treatment of human psoriasis. It has been suggested that dysregulation of normal AHR function could be important in the pathogenesis of chronic skin disease with aberrant epidermal differentiation, psoriasis and atopic dermatitis.

Some polymorphisms in AHR have been reported to affect the functionality of the receptor by either inducing or inhibiting its ligand-dependent activation. The polymorphism in AHR rs2066853 AA homozygotes is associated with significantly lower mRNA expression of AHR, the aryl hydrocarbon receptor nuclear translocator (ARNT), and cytochrome P4501B1 (CYP1B1) and is thought to reduce AHR activity and decrease metabolism regulated by cytochrome p450. The present study showed that AHR rs2066853 AA homozygotes were not likely to develop psoriasis. If this finding was due to reduced AHR activity, this result would be consistent with previous studies. It remains to be clarified what the direct targets of AHR are and how these targets are linked to the inflammatory networks that are affected. The association of AHR polymorphism with PV has not been previously reported and therefore whether the polymorphism in AHR rs2066853 AA homozygotes in HLA-C*06:02 positive PV patients is important in the AHR regulation of such immune cells and cytokines remains to be established.

This study reported that an AHR SNP associated with psoriasis susceptibility according to the HLA-C*06:02 gene. SNPs in the α-helix coiled-coil rod homologue (HCR) gene have also been reported to be associated with psoriasis susceptibility. HCR is located at 110 kb telomeric to the HLA-C region, and within PSORS1. In the PSORS1 locus, strong linkage disequilibrium between genes has made it difficult to distinguish the effects of the nearby genes. Thus, it is possible that HLA-C might be the main candidate gene of PSORS1. AHR is not located within PSORS1, and is located on chromosome 7. Thus, AHR does not seem to be associated with the linkage disequilibrium between other candidate genes for psoriasis susceptibility. Further studies of AHR association in psoriasis patients are needed.

It has been suggested that the fungus Malassezia is associated with the development of psoriasis. Recent studies of the skin microbiome in Japanese patients with psoriasis that were carried out in our laboratory, indicated that the microbiome in patients was independent from that in healthy controls. A similar study in Japanese patients with psoriasis also identified Malassezia restricta as the most abundant skin-resident fungal species. Malassezin, a ligand of AHR, is uniquely produced by Malassezia, and may play a crucial role in skin homeostasis and in the development of psoriasis. Antifungal treatment may therefore result in the treatment of psoriasis by modulating the effects on the immune system that are induced by the AHR.

In conclusion, the results from the present study do not conclusively prove that AHR polymorphisms serve as a factor in determination of susceptibility to PV. However, in HLA- C*06:02-positive PV patients, rs2066853 AA homozygotes were significantly decreased compared to HLA- C*06:02-negative PV patients. Of the two AHR polymorphisms studied, the AHR rs2066853 AA homozygote may thus be a gene allele that is protective against PV.

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Conflict of Interest

The authors declare no conflict of interest.

References

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