



Frequencies of human leukocyte antigen alleles and haplotypes among Japanese patients with age-related macular degeneration

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Abstract

Purpose Stem cell therapy is a potential treatment for retinal disorders. We are currently exploring treating HLA matched patients of age-related macular degeneration (AMD) by using allogenic retinal pigment epithelium cells derived from induced pluripotent stem cells (iPS-RPE) from human leukocyte antigen (HLA) homozygote donors. The purpose of this study was to investigate the frequency of HLA class I and II alleles and haplotypes in Japanese patients with AMD.

Study design Cross-sectional observation clinical study.

Methods A total of 138 consecutive patients diagnosed with neovascular AMD (mean age, 76.0 ± 7.8 years, 105 men) and 300 controls were included in the study. The frequencies of HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 alleles were determined using illumina MiSeq platform. Frequencies of HLA alleles at six loci in patients with AMD were compared with those of the controls.

Results The alleles with the highest prevalence at each locus were A*24:02 (29.7%), B*52:01 (15.5%), C*12:02 (16.1%), DRB1*09:01 (19.1%), DQB1*06:01 (23.2%), and DPB1*05:01 (40.5%). There were no significant associations between the HLA alleles and AMD. The most common haplotype was A*24:02-B*52:01-C*12:02-DRB1*15:02-DQB1*06:01-DPB1*09:01, with a 9.8% genetic frequency among all haplotypes, detected in 18.8% of the patients.

Conclusion The genotype of HLA in patients with AMD was not different from that in the Japanese control population. Thus, therapy with iPS-RPE of the most frequent HLA haplotype could be a feasible alternative for AMD in a wider population.

Keywords Age-related macular degeneration · Haplotype frequency · Human leukocyte antigen · Japan · Induced pluripotent stem cells

Introduction

Age-related macular degeneration (AMD) is a common disease and with the rising life span has become a global health concern [1]. Intravitreal injection of anti-vascular endothelial growth factor (VEGF) is currently the first-line therapy for AMD, with evidence of efficacy and safety based on large clinical trials [2, 3]. However, once the neural retina or retinal pigment epithelium (RPE) degenerates with disease progression, current therapies show limited efficacy; in recent years, increasing attention has been given to regenerative stem cell therapy to replace atrophic retinal components.

Accumulated damage to the RPE plays an important role in AMD pathogenesis [4]. Several sources including allogenic fetal RPE and autologous peripheral RPE are reported as potential cell sources for damage repair [5, 6], however, these cells have limitations, such as immune rejection,

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complicated invasive surgery, and insufficient cell volume preparation. Embryonic stem cells (ESCs), first cultured in 1998 by Thomson et al. [7], provide a new approach to generating an unlimited supply of RPE cells for replacement therapies [8]. Clinical trials using RPE cell suspensions derived from ESCs for advanced dry AMD are reported [9]. However, utilizing ESCs involves ethical problems and requires administration of immune-suppressants, which can increase the risk of complications in elderly patients. Therefore, induced pluripotent stem cells (iPSCs) have been proposed as an alternative source of donor cells [10]. Recently, we reported the first clinical case using human iPSCs, in which we transplanted autologous iPSC-derived RPE cell sheets into a patient with neovascular AMD [11]. Autologous iPSCs are an ideal source; however, the production of high-quality cells in a sufficient amount is costly and time-consuming. To resolve these issues, we are now conducting a new clinical trial using allogenic iPSCs with a human leukocyte antigen (HLA)-matched donor from iPSC banking [12].

The HLA genes encode the six major antigen proteins (HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1), which play important roles in graft rejection for stem cell therapy, and thus HLA matching between donors and recipients reduces the risk of rejection in hematopoietic stem cell transplantation [13]. However, before this approach can be tested for AMD, it is necessary to determine the frequencies of HLA alleles and haplotypes in Japanese patients with AMD, and the possibility of matching with healthy donors. Therefore, the present cross-sectional observational study was conducted to investigate the frequencies of HLA alleles at six loci (HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1) using next-generation sequencing (NGS) methods, and to estimate the haplotype frequencies in Japanese patients with AMD. Moreover, we investigated the potential association between exudative AMD and HLA alleles through a comparison with the reported HLA allele frequencies of the general Japanese population.

Patients and methods

Patients

The Kobe City Medical Center General Hospital ethics committee reviewed and approved the protocol for this cross-sectional observational study. This study conformed to the tenets of the Declaration of Helsinki.

After written informed consent, consecutive patients with exudative AMD who received anti-VEGF treatment were recruited from the ophthalmology clinic of Kobe City Medical Center General Hospital from September 2015 to September 2016. The disease was classified into typical age-related macular degeneration (t-AMD), polypoidal choroidal vasculopathy

(PCV), retinal angiomatous proliferation (RAP), and myopic CNV (mCNV). The decision to initiate anti-VEGF treatment was based on complete ocular examinations, including best-corrected decimal visual acuity measurement, slit-lamp biomicroscopy, dilated funduscopy, and spectral domain-optical coherence tomography.

Data for 300 healthy control subjects were obtained from the HLA Foundation Laboratory.

A 7-mL peripheral blood sample was obtained from each patient, and DNA was then extracted using the salting-out method and stored at -20°C . DNA samples were obtained from peripheral lymphocyte buccal cells using a Wizard Genomic DNA Purification Kit (Promega) according to the manufacturer's guidelines.

Determination of HLA alleles

All subjects were genotyped at the HLA Foundation Laboratory using NGS high-resolution HLA typing for HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1. The NGS HLA typing was performed based on the protocol of commercially available kits (Seisco Genetics) with Illumina MiSeq technology. In brief, the sequencing involved consecutive PCR reactions with bar codes incorporated to track individual samples followed by application to the MiSeq platform for NGS.

Determination of haplotypes

The haplotype frequencies were calculated using the haplo.em program, evaluated by HAPLO.STATS (version 1.6.0 provided in the public domain by Mayo clinic <http://www.mayo.edu/research/documents/manualhaplostats/doc-20167217>.) software operated in the R language.

Statistical analyses

The alleles at each loci were checked for deviations from the Hardy–Weinberg equilibrium based on a comparison of the observed frequency to the expected frequency (a P value ≥ 0.05 indicated no significant deviation).

We used a case–control study design to investigate the odds' ratios (ORs) for the associations of HLA alleles and haplotype frequencies with AMD. ORs and 95% confidence intervals of each allele were calculated using logistic and linear regression. We further tested whether there was a significant trend between the number of alleles possessed and the risk of AMD using the Wald test in plink version 1.9 software [14].

Results

One hundred and thirty-eight patients with AMD were included in the study and genotyped at six HLA loci. All patients were Japanese; 105 were men and 33 were women. The disease groups of patients were t-AMD (40 patients), PCV (80 patients), RAP (8 eyes), and mCNV (2 patients), and seven cases could not be classified because of their advance appearance with unknown course and one case could not be classified due to allergy to fluorescein. The mean age of the patients was 76.05 ± 7.81 years at the time of HLA examination.

The HLA class I (HLA-A, -B, -C) allele frequencies are shown in Table 1. Overall, we identified 15 HLA-A, 31 HLA-B, and 18 HLA-C alleles. The alleles with the highest prevalence at each locus were A*24:02 (30.8%), B*52:01 (15.6%), and C*12:02 (16.3%).

The frequencies of HLA class II alleles (HLA-DRB1, -DQB1, -DPB1) are shown in Table 2. We identified 24 HLA-DRB1, 13 HLA-DQB1, and 11 HLA-DPB1 alleles. The alleles with the highest prevalence at each locus were DRB1*09:01 (18.5%), DQB1*06:01 (21.4%), and DPB1*05:01 (42.4%).

In the locus level analysis (Tables 1 and 2), we found that three alleles (B*46:01, DRB1*04:05, and DQB1*04:01) had a *P* value of less than 0.05. However, there were no significant associations of any HLA allele with AMD after adjustment for multiple comparisons using the Bonferroni method ($P=0.0005$).

Table 3 lists the ten most common six-locus haplotypes comprising the HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 frequencies detected in the patients with AMD. The most common haplotype was identified as A*24:02-B*52:01-C*12:02-DRB1*15:02-DQB1*06:01-DPB1*09:01, with a 9.8% genetic frequency among all haplotypes, which was detected in 18.8% of the patients. Comparing the disease's types, the most common six locus haplotype was 20% in t-AMD patients and 21.3% in PCV patients.

Table 4 lists the ten most common three-locus haplotypes comprising the HLA-A, -B and -DRB1 frequencies, considered important in the rejection mechanism of transplant treatment in other organs [15], detected in the patients with AMD. The most common haplotype was identified as A*24:02-B*52:01-DRB1*15:02, with a 12.0% genetic frequency among all haplotypes, which was detected in 23.2% of the patients. In comparison between disease types, the most common three-locus haplotype was 20% of t-AMD and 22.5% of PCV.

Discussion

The genetic frequencies of HLA-A, -B, -C, -DRB1, -DPB1, and -DQB1 alleles elucidated in the AMD group were not significantly different from the control group, and were

similar to that of previous population study reports. Moreover, we found that the most common haplotypes of six and three loci in patients with AMD were A*24:02-B*52:01-C*12:02-DRB1*15:02-DQB1*06:01-DPB1*09:01 and A*24:02-B*52:01-DRB1*15:02, which was consistent with previous population study reports [16–18]. Even in observation by group, the frequency of most common of six and three loci in t-AMD and PCV patients, which are the majority among AMD patients, were similar.

We reported an AMD case with autologous iPSC–RPE transplantation [11] and we believe that individualized iPSC-derived tissues are the ideal transplant cells, with lower possibility of transplant rejection; however, because of their high cost, the strategy does not seem practical as a standard treatment. The alternative to personalized iPSC therapy is to use HLA consensus to select and transplant so as to minimize the risk of allograft rejection. One approach is to create iPSC banks with limited cell donors of homozygous HLA types that are consistent with frequent HLA types, to create a large pool to treat potentially HLA-matched recipients. In our results, nearly 19% of patients with AMD were eligible for the transplantation of the iPSC–RPE line of the most frequent HLA type with a 6-loci match, as were 23% of the patients with AMD with a 3-loci match.

To test the feasibility of HLA-matched transplantation, Sugita et al. transplanted iPSC-derived RPEs of homozygous major histocompatibility complexes (MHCs) and observed no signs of rejection in the MHC-matched monkeys, whereas a substantial immune attack was observed around the graft accompanied by retinal tissue damage in the unmatched model [19]. Moreover they also report that T-cells did not respond to HLA-A, -B, and -DRB1-matched iPSC-derived RPE cells from HLA homozygote donors [20]. Based on these findings, we hypothesized that iPSC–RPE from MHC homozygous donors could be used to treat retinal diseases in histocompatible recipients.

Recently, we started a new clinical study to evaluate the possibility of the transplantation of allogenic iPSCs-derived RPE cells in patients with AMD using iPSCs with the most frequent HLA homozygous genotype, supplied by the Center for iPSC Research and Application (CiRA) iPSC bank [21]. The CiRA iPSC bank has established cell lines from the blood and skin of healthy volunteers with HLA homozygous genotypes common in the Japanese population. This has been made possible by collaborating with the bone marrow bank project to screen thousands of potential stem cell donors. Currently, we are investigating whether 6-loci HLA matching in iPSC–RPE cells can help avoid rejection in patients with AMD.

HLA alleles show strong linkage disequilibrium, and with a specific disease-susceptible allele the haplotype frequency of patients with AMD may differ from that of the typical Japanese population. Therefore, we also investigated

Table 1 HLA-A, -B, -C, allele frequencies in AMD patients and control

Target_allele	Allele frequency		Association(logistic)			
	Case	Control	OR	OR 95% CI	OR 95% CI	<i>P</i>
A*01:01	0.72	0.17	4.40	0.40	48.91	0.23
A*02:01	13.04	11.67	1.14	0.74	1.78	0.55
A*02:03	0.00	0.17	NA	NA	NA	NA
A*02:06	9.78	7.67	1.32	0.79	2.19	0.29
A*02:07	4.71	2.67	1.85	0.86	3.95	0.11
A*02:10	0.00	0.50	NA	NA	NA	NA
A*02:18	0.36	0.00	NA	NA	NA	NA
A*02:28	0.00	0.17	NA	NA	NA	NA
A*03:01	0.36	0.00	NA	NA	NA	NA
A*03:02	0.00	0.17	NA	NA	NA	NA
A*11:01	9.42	9.83	0.95	0.59	1.54	0.85
A*11:02	0.36	0.50	0.72	0.07	7.01	0.78
A*24:02	31.52	36.50	0.80	0.59	1.09	0.15
A*24:07	0.00	0.17	NA	NA	NA	NA
A*24:20	0.72	0.67	1.09	0.20	6.01	0.92
A*26:01	9.78	8.00	1.27	0.76	2.12	0.37
A*26:02	2.17	2.33	0.93	0.35	2.47	0.88
A*26:03	0.72	2.83	0.24	0.06	1.08	0.06
A*26:05	0.00	0.33	NA	NA	NA	NA
A*31:01	10.51	7.50	1.44	0.88	2.33	0.14
A*33:03	5.80	8.17	0.68	0.37	1.24	0.21
B*07:02	4.71	5.67	0.83	0.43	1.58	0.57
B*13:01	1.45	1.50	0.97	0.29	3.19	0.95
B*13:02	0.00	0.17	NA	NA	NA	NA
B*15:01	6.52	7.17	0.90	0.51	1.60	0.73
B*15:07	0.36	0.67	0.54	0.06	4.88	0.58
B*15:11	2.90	1.33	2.25	0.83	6.12	0.11
B*15:18	1.45	1.83	0.78	0.25	2.51	0.68
B*15:27	0.36	0.67	0.54	0.06	4.88	0.58
B*15:93	0.00	0.17	NA	NA	NA	NA
B*27:04	0.36	0.00	NA	NA	NA	NA
B*35:01	8.33	9.83	0.83	0.50	1.38	0.47
B*35:05	0.00	0.17	NA	NA	NA	NA
B*37:01	0.72	0.50	1.46	0.24	8.81	0.68
B*38:02	0.00	0.33	NA	NA	NA	NA
B*39:01	2.17	2.17	1.00	0.37	2.70	0.99
B*39:02	0.36	0.17	2.18	0.14	35.15	0.58
B*39:04	0.36	0.00	NA	NA	NA	NA
B*40:01	4.35	4.83	0.90	0.46	1.76	0.76
B*40:02	6.88	9.33	0.71	0.41	1.23	0.22
B*40:03	1.45	0.50	2.96	0.65	13.39	0.16
B*40:06	4.35	4.17	1.05	0.51	2.15	0.90
B*44:02	0.36	0.00	NA	NA	NA	NA
B*44:03	4.35	6.50	0.66	0.34	1.27	0.21
B*46:01	6.88	3.33	2.14	1.12	4.10	0.02
B*48:01	3.99	2.00	2.08	0.89	4.84	0.09
B*51:01	11.23	8.83	1.32	0.82	2.12	0.26
B*51:02	0.36	0.17	2.18	0.14	35.15	0.58
B*51:03	0.00	0.17	NA	NA	NA	NA

Table 1 (continued)

Target_allele	Allele frequency		Association(logistic)			
	Case	Control	OR	OR 95% CI	OR 95% CI	<i>P</i>
B*52:01	15.58	12.33	1.33	0.88	2.01	0.18
B*54:01	4.35	7.83	0.52	0.27	1.01	0.05
B*55:02	1.81	3.17	0.56	0.20	1.52	0.25
B*55:04	0.36	0.17	2.18	0.14	35.15	0.58
B*56:01	0.72	0.67	1.09	0.20	6.01	0.92
B*56:03	0.00	0.17	NA	NA	NA	NA
B*58:01	1.09	0.50	2.20	0.44	11.04	0.34
B*59:01	0.72	1.50	0.48	0.10	2.23	0.35
B*67:01	1.09	1.50	0.72	0.19	2.70	0.62
C*01:02	13.41	16.50	0.77	0.51	1.18	0.23
C*01:03	1.09	0.17	6.64	0.68	64.46	0.10
C*03:02	1.09	0.50	2.20	0.44	11.04	0.34
C*03:03	16.30	14.50	1.16	0.77	1.72	0.48
C*03:04	10.51	12.83	0.81	0.52	1.26	0.35
C*04:01	3.99	4.50	0.88	0.42	1.82	0.72
C*04:82	0.36	0.00	NA	NA	NA	NA
C*05:01	0.36	0.00	NA	NA	NA	NA
C*06:02	0.72	0.67	1.09	0.20	6.01	0.92
C*07:02	9.78	12.00	0.80	0.51	1.27	0.35
C*07:04	0.36	1.33	0.27	0.03	2.15	0.21
C*08:01	6.52	7.50	0.87	0.50	1.51	0.61
C*08:03	2.17	1.17	1.90	0.63	5.77	0.26
C*08:22	0.72	0.00	NA	NA	NA	NA
C*12:02	16.30	12.33	1.40	0.93	2.10	0.11
C*14:02	9.42	7.00	1.39	0.83	2.34	0.21
C*14:03	3.99	6.50	0.59	0.30	1.18	0.14
C*15:02	2.90	2.50	1.17	0.48	2.83	0.73

HLA Human leukocyte antigen, *AMD* age-related macular degeneration, *OR* odds ratio, *CI* confidence interval

the potential relationship between HLA alleles and AMD; however, we found no statistically significant associations after adjustment for multiple comparisons.

The HLA loci on chromosome 6 (6p21.3) are known as the most polymorphic regions within the human genome, and are critical for regulation of the immune response. Many HLA alleles are reported as associated with specific immunological diseases [22], and evidence suggests a potential relationship between HLA and AMD pathology. Inflammation plays an important role in AMD development and progression; indeed, drusen, one diagnostic factor for AMD, is composed of inflammatory proteins, and HLA class II immunoreactivity has been observed in human retinas affected by AMD [23, 24].

Several reports demonstrate a relationship between HLA alleles and AMD. Goverdhan et al. [25] first reported an association between HLA genotypes and AMD, in which the HLA-C*07:01 allele increases AMD risk, whereas B*40:01 and DRB1*13:01 are negatively associated with AMD risk.

They also report that the HLA-C*0701 allele, in combination with the inhibitory killer immunoglobulin-like receptor (KIR) centromeric-AA haplotype, is associated with AMD [26]. Furthermore, Becerril et al. [27] report that the HLA-B*27 allele, but not HLA-A or HLA-DRB1, is positively correlated with AMD.

There are several potential reasons for the discrepancy between our results and those reported to date on the association of HLA and AMD. First, our study included a relatively small number of subjects, which could lead to both false-positive and false-negative associations because of high rates of HLA polymorphism. In practice, a two-step genotyping procedure should be performed, which would help reduce the number of multiple comparisons to further minimize the Type I error rate [25]. Second, the previous reports focused on European populations in the UK [25] and the southern Mediterranean [27]. Given that HLA diversity shows race-specificity, our results might suggest a different pattern for the Japanese population, which requires further

Table 2 HLA-DRB1, -DQB1, -DPB1 allele frequencies in patients with AMD and controls

Target_allele	Allele frequency		Association(logistic)			
	Case	Control	OR	OR 95% CI	OR 95% CI	<i>P</i>
DPB1*02:01	21.17	23.17	0.89	0.63	1.26	0.51
DPB1*02:02	2.92	4.17	0.70	0.31	1.56	0.38
DPB1*03:01	4.38	5.67	0.77	0.40	1.50	0.44
DPB1*04:01	4.38	4.00	1.09	0.55	2.16	0.80
DPB1*04:02	8.76	10.00	0.87	0.53	1.42	0.57
DPB1*05:01	42.70	38.33	1.19	0.89	1.58	0.23
DPB1*06:01	0.73	0.50	1.47	0.24	8.88	0.68
DPB1*09:01	11.68	10.17	1.18	0.74	1.88	0.49
DPB1*135:01	0.37	0.00	NA	NA	NA	NA
DPB1*13:01	0.37	1.50	0.24	0.03	1.90	0.18
DPB1*14:01	2.19	1.33	1.67	0.57	4.92	0.35
DPB1*17:01	0.00	0.17	NA	NA	NA	NA
DPB1*19:01	0.00	0.33	NA	NA	NA	NA
DPB1*20:01	0.00	0.17	NA	NA	NA	NA
DPB1*25:01	0.00	0.17	NA	NA	NA	NA
DPB1*36:01	0.37	0.00	NA	NA	NA	NA
DPB1*41:01	0.00	0.33	NA	NA	NA	NA
DQB1*02:02	0.00	0.17	NA	NA	NA	NA
DQB1*03:01	14.86	13.33	1.14	0.75	1.73	0.54
DQB1*03:02	7.25	9.67	0.73	0.43	1.24	0.24
DQB1*03:03	19.57	15.17	1.35	0.93	1.95	0.11
DQB1*03:13	0.36	0.00	NA	NA	NA	NA
DQB1*03:19	0.00	0.17	NA	NA	NA	NA
DQB1*04:01	7.97	14.00	0.53	0.32	0.87	0.01
DQB1*04:02	5.07	5.33	0.95	0.50	1.81	0.87
DQB1*05:01	5.44	7.50	0.72	0.39	1.30	0.27
DQB1*05:02	1.81	2.83	0.65	0.24	1.74	0.39
DQB1*05:03	3.99	2.50	1.65	0.74	3.68	0.23
DQB1*06:01	21.38	16.83	1.37	0.94	1.98	0.10
DQB1*06:02	8.33	6.33	1.35	0.78	2.31	0.28
DQB1*06:03	0.00	0.17	NA	NA	NA	NA
DQB1*06:04	3.62	5.33	0.68	0.33	1.38	0.29
DQB1*06:09	0.36	0.67	0.54	0.06	4.88	0.58
DRB1*01:01	4.75	6.83	0.68	0.36	1.29	0.24
DRB1*04:01	0.37	1.67	0.21	0.03	1.68	0.14
DRB1*04:03	1.83	3.50	0.50	0.19	1.36	0.18
DRB1*04:04	0.37	0.50	0.73	0.08	7.06	0.78
DRB1*04:05	8.76	14.00	0.57	0.35	0.94	0.03
DRB1*04:06	1.83	3.00	0.59	0.22	1.63	0.31
DRB1*04:07	0.73	0.17	4.43	0.40	49.27	0.23
DRB1*04:10	2.92	2.00	1.49	0.59	3.73	0.40
DRB1*07:01	0.00	0.17	NA	NA	NA	NA
DRB1*08:02	4.38	5.50	0.79	0.40	1.55	0.49
DRB1*08:03	8.03	6.17	1.33	0.77	2.30	0.31
DRB1*09:01	18.61	14.50	1.33	0.92	1.93	0.13
DRB1*10:01	0.37	0.50	0.73	0.08	7.06	0.78
DRB1*11:01	3.65	2.33	1.61	0.70	3.72	0.27
DRB1*11:08	0.00	0.17	NA	NA	NA	NA
DRB1*12:01	4.75	4.33	1.09	0.56	2.12	0.79

Table 2 (continued)

Target_allele	Allele frequency		Association(logistic)			
	Case	Control	OR	OR 95% CI	OR 95% CI	P
DRB1*12:02	2.56	1.83	1.42	0.54	3.73	0.48
DRB1*13:01	0.00	0.17	NA	NA	NA	NA
DRB1*13:02	4.02	6.17	0.64	0.33	1.27	0.21
DRB1*14:02	0.37	0.00	NA	NA	NA	NA
DRB1*14:03	1.83	1.67	1.10	0.37	3.28	0.87
DRB1*14:05	2.19	1.17	1.92	0.63	5.82	0.25
DRB1*14:06	1.10	1.67	0.65	0.18	2.40	0.52
DRB1*14:54	2.56	3.17	0.80	0.33	1.94	0.62
DRB1*15:01	9.49	6.67	1.48	0.88	2.49	0.14
DRB1*15:02	13.50	11.17	1.25	0.81	1.93	0.32
DRB1*16:02	1.10	1.00	1.10	0.27	4.45	0.90

HLA Human leukocyte antigen, *AMD* age-related macular degeneration, *OR* odds ratio, *CI* confidence interval

Table 3 HLA-A-B-C-DRB1-DQB1-DPB1 haplotype frequencies in patients with AMD

Rank	Haplotype	Phenotype frequency	Genetic frequency
1	A*24:02-B*52:01-C*12:02-DRB1*15:02-DQB1*06:01-DPB1*09:01	18.84	9.78
2	A*24:02-B*07:02-C*07:02-DRB1*01:01-DQB1*05:01-DPB1*04:02	5.80	2.90
3	A*26:01-B*40:02-C*03:04-DRB1*09:01-DQB1*03:03-DPB1*05:01	3.62	1.81
4	A*02:07-B*46:01-C*01:02-DRB1*09:01-DQB1*03:03-DPB1*05:01	2.90	1.45
5	A*33:03-B*44:03-C*14:03-DRB1*13:02-DQB1*06:04-DPB1*04:01	2.90	1.45
6	A*02:07-B*46:01-C*01:02-DRB1*08:03-DQB1*06:01-DPB1*02:01	2.90	1.45
7	A*24:02-B*52:01-C*12:02-DRB1*15:02-DQB1*06:01-DPB1*05:01	2.17	1.09
8	A*11:01-B*15:01-C*03:03-DRB1*09:01-DQB1*03:03-DPB1*05:01	2.17	1.09
9	A*11:01-B*48:01-C*08:03-DRB1*15:01-DQB1*03:01-DPB1*05:01	2.17	1.09
10	A*24:02-B*51:01-C*14:02-DRB1*09:01-DQB1*03:03-DPB1*05:01	2.17	1.09

HLA Human leukocyte antigen, *AMD* age-related macular degeneration

Table 4 HLA-A-B-DRB1 haplotype frequencies in patients with AMD

Rank	Haplotype	Phenotype frequency	Genetic frequency
1	A*24:02-B*52:01-DRB1*15:02	23.19	11.96
2	A*26:01-B*40:02-DRB1*09:01	6.52	3.26
3	A*02:07-B*46:01-DRB1*08:03	5.07	2.54
3	A*24:02-B*07:02-DRB1*01:01	5.07	2.54
5	A*02:07-B*46:01-DRB1*09:01	3.62	1.81
5	A*33:03-B*44:03-DRB1*13:02	3.62	1.81
7	A*31:01-B*51:01-DRB1*08:02	2.90	1.45
8	A*11:01-B*15:01-DRB1*04:06	2.17	1.09
8	A*11:01-B*48:01-DRB1*15:01	2.17	1.09
8	A*24:02-B*51:01-DRB1*09:01	2.17	1.09
8	A*24:02-B*54:01-DRB1*04:05	2.17	1.09
8	A*24:20-B*55:02-DRB1*04:05	2.17	1.09
8	A*02:01-B*13:01-DRB1*12:02	1.45	0.72

HLA Human leukocyte antigen, *AMD* age-related macular degeneration

investigation [28]. The third reason may reflect differences in the analytical methods used among the studies. In the present study, an NGS method was used instead of a conventional genotyping method. Pappas et al. also used NGS methods and report the lack of statistically significant associations for any of the HLA class II loci, including DRB1*13:01, with AMD. This consistency with our results might suggest that the NGS method could cover the HLA region more exhaustively, allowing for more detailed discrimination despite the high rate of polymorphisms.

Overall, these preliminary data suggest the feasibility of iPSC-derived RPE cell transplantation based on the iPSC bank system. Determination of the safety and usefulness of transplantation therapy using HLA-matched iPSCs would provide an extremely valuable alternative to RPE cell transplantation as a future treatment option for AMD.

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