Ⅴ　平成23年度班会議プログラム

平成23年度班会議プログラム（平成23年12月9日　東京ステーションコンファレンス）

	9:00- 9:10	厚生労働省健康局疾病対策課長挨拶	課長代行　眞野 訓先生
	9:10- 9:40	班全体およびSS分科会統括	住田孝之
	9:40-10:00	SLE/ AOSD分科会統括	山本一彦
	10:00-10:20	PM/DM分科会統括	上阪　等
	10:20-11:50	SS分科会研究報告	座長　住田孝之
1	10:20-10:30	M3Rを分子標的とした自己免疫性唾液腺炎に関する研究 筑波大学医学医療系（膠原病・リウマチ・アレルギー）　住田孝之	
2	10:30-10:40	シェーグレン症候群の病変局所における Th サブセットの局在 九州大学大学院歯学研究院 口腔顎顔面病態学講座 顎顔面腫瘍制御学　中村誠司	
3	10:40-10:50	シェーグレン症候群唾液腺上皮細胞におけるToll-like receptor3による細胞死調節シグナルとAktの発現に関する研究 長崎大学大学院医歯薬学総合研究科医療科学専攻展開医療科学　川上　純	
4	10:50-11:00	シェーグレン症候群におけるダイオキシンを介したEBV再活性化の関与 鶴見大学　歯学部　斎藤一郎	
5	11:00-11:10	シェーグレン症候群における口腔内病変と唾液中EGFの関係に関する研究 兵庫医科大学リウマチ・膠原病科　佐野　統	
6	11:10-11:20	シェーグレン症候群における 唾液分泌量試験の妥当性に関する研究 金沢医科大学血液免疫内科　梅原久範	
7	11:20-11:30	シェーグレン症候群診断基準におけるドライアイに関する研究 東京女子医科大学医学部医学科眼科　高村悦子	
8	11:30-11:40	シェーグレン症候群の国際統一基準の比較検討に関する研究 慶應義塾大学医学部　眼科　坪田一男	
9	11:40-11:50	シェーグレン症候群の診断基準の検証 筑波大学医学医療系内科（膠原病・リウマチ・アレルギー）　坪井洋人	
	11:50-12:30	昼　食	

12:30–14:10　SLE/AOSD分科会研究報告　座長　山本一彦

1　12:30–12:40　全身性エリテマトーデス患者血清における可溶性LAG3濃度に関する研究
東京大学アレルギー・リウマチ内科　山本一彦

2　12:40–12:50　SLE/ASODの遺伝因子解析に関する研究
京都大学大学院医学研究科　山田　亮

3　12:50–13:00　Fcγレセプター IIB欠損マウスにおけるYaa遺伝子変異の与える影響
順天堂大学　膠原病内科　天野浩文

4　13:00–13:10　B細胞免疫寛容破綻におけるFcγRIIB発現欠損とSlam遺伝子多型との相補作用
順天堂大学大学院医学研究科分子病理病態学　広瀬幸子

5　13:10–13:20　全身性エリテマトーデスにおけるMAIT細胞の解析
(独)国立精神・神経医療研究センター神経研究所免疫研究部　三宅幸子

6　13:20–13:30　angiotensin converting enzyme 2を阻害する自己抗体の病原性に関する研究
国立国際医療研究センター　三森明夫

7　13:30–13:40　リウマチ性疾患に伴う腎障害におけるポドサイト障害の研究
埼玉医科大学病院　リウマチ膠原病科　三村俊英

8　13:40–13:50　B 細胞を標的とした全身性エリテマトーデスの治療の開発に関する研究
産業医科大学医学部第一内科学講座　田中良哉

9　13:50–14:00　全身性エリテマトーデス難治性病態の治療標的分子探索に関する研究
慶應義塾大学医学部リウマチ内科　竹内　勤

10　14:00–14:10　抗リン脂質抗体症候群における血栓傾向のメカニズムに関する研究
北海道大学大学院医学研究科　内科学講座・第二内科　渥美達也

14:10–14:30　コーヒーブレイク

14:30–16:10　PM/DM分科会研究報告　座長　上阪　等

1　14:30–14:40　自己免疫性筋炎における再生筋線維の役割に関する研究

東京医科歯科大学　膠原病・リウマチ内科　上阪　等

2　14:40–14:50　炎症性筋疾患における疾患感受性遺伝子の検索

東京女子医科大学リウマチ科　川口鎮司

3　14:50–15:00　皮膚筋炎におけるmicroRNA解析

熊本大学大学院生命科学研究部皮膚病態治療再建学　神人正寿

4　15:00–15:10　筋無症候性皮膚筋炎および抗MDA5抗体陽性皮膚筋炎に関する臨床疫学

名古屋大学大学院医学系研究科　皮膚結合組織病態学　室　慶直

5　15:10–15:20　皮膚筋炎の病態解明に向けてのヒト筋肉由来血管内皮細胞株樹立の試み

山口大学大学院医学系研究科　神経内科　神田　隆

6　15:20–15:30　筋炎に合併する難治性間質性肺炎の診断と治療に関する研究

京都大学大学院医学研究科内科学講座臨床免疫学　三森経世

7　15:30–15:40　皮膚筋炎の基礎・臨床研究／皮膚筋炎の診断・治療指針の作成に関する研究

金沢大学医薬保健研究域医学系皮膚科学　藤本　学

8　15:40–15:50　多発筋炎/皮膚筋炎の病態と治療法解明のための統合的検討

東京大学医学部附属病院神経内科　清水潤

9　15:50–16:00　多発筋炎/皮膚筋炎の疫学調査（1）－臨床調査個人票の解析から入力率と基本疫学特性－

埼玉医科大学医学部公衆衛生学　太田晶子

10　16:00–16:10　多発筋炎/皮膚筋炎の疫学調査（2）－臨床調査個人票の解析から臨床疫学特性－

東京医科歯科大学大学院　脳神経病態学　冨満弘之

16:10–16:20　閉会の辞（事務連絡）

VI　研究成果刊行物・別刷

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ORIGINAL ARTICLE

A trans-ethnic genetic study of rheumatoid arthritis identified *FCGR2A* as a candidate common risk factor in Japanese and European populations

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Abstract Rheumatoid arthritis (RA) is a common systemic autoimmune disease and its onset and prognosis are controlled by genetic, immunological, and environmental factors. The *HLA* locus, particularly *HLA-DRB1*, is its strongest genetic risk determinant across ethnicities. Several other genes, including *PTPN22* and *PADI4*, show modest association with RA. However, they cover only a part of its genetic components and their relative contribution is different between populations. To identify novel genetic determinants, we took a candidate gene approach in a trans-ethnic manner. After critical selection of 169 genes based on their immunological function, we performed SNP discovery of these genes by the resequencing of exons and surrounding areas using European and Japanese DNAs. We then generated a panel of 1,509 SNPs for case–control association study in both populations. The DerSimonian–Laird test for meta-analysis, using the combined results of the two populations, identified rs7551957 at the 5′-flanking region of the low-affinity Fc-gamma receptor IIa (*FCGR2A*) gene as the strongest candidate for the association ($p = 8.6 \times 10^{-5}$, odds ratio = 1.58 with 95%CI 1.25–1.99). Suggestive signals were also obtained for three SNPs in the dihydropyrimidine dehydrogenase (*DPYD*) gene (rs6685859; $p = 1.3 \times 10^{-4}$, rs7550959; $p = 1.5 \times 10^{-4}$ and rs7531138; $p = 1.7 \times 10^{-4}$) and an intronic SNP, rs2269310, of the erythrocytic spectrin beta (*SPTB*) gene ($p = 7.9 \times 10^{-4}$).

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Introduction

Rheumatoid arthritis (RA [OMIM: 180300]) is a systemic autoimmune disease [1], and is one of the most common forms of inflammatory arthritis, affecting up to 1% of the adult population [2]. Symptoms are chronic, destructive, and debilitating arthritis with a variation in the number of clinical features, such as the presence of autoantibody and joint erosions [3]. Clinical manifestation of RA is related to the development of a variety of autoantibodies, including antibodies to citrullinated peptide antigens and rheumatoid factor, although their pathological role is still unclear [4, 5].

Compelling evidence from genome-wide association (GWA) studies demonstrated that the *HLA* locus is the strongest genetic determinant beyond ethnicity [6]. However, the *HLA* locus contributes to only approximately 37% of the overall genetic susceptibility [7], suggesting the presence of other genes that are genetically associated with pathogenesis, clinical phenotype, and disease heterogeneity. Moreover, the relative contribution of RA-related genes is considered to be different between ethnicities. Indeed, the *PTPN22* gene was shown to be associated with RA in populations of European descent, but not in Asians [8]. Similarly, the *PADI4* gene showed a strong association with RA in Asians [9], but the association was much weaker in Europeans. Intronic SNPs in the *STAT4* gene were identified to be strongly associated with RA in Europeans [10], and their modest statistical association was confirmed in Asians [11], providing the first example of non-*HLA* RA-associated genes in two major ethnicities.

Importantly, however, very few hypothesis-independent GWA studies have succeeded in the identification of non-*HLA* genes associated with RA. A genome scan by the Wellcome Trust study was only able to successfully identify *HLA* and *PTPN22* loci as genome-wide significant [6]. This may be due to the disease heterogeneity with phenocopies, and the insufficient power to detect genes with modest effects [12]. Indeed, SNPs associated with RA in non-*HLA* genes showed only intermediate allelic odds ratios (ORs) of 1.3–2.0. To overcome such shortcomings, studies need to be enlarged, enrolling several thousand patients and a similar number of controls. Alternatively, a candidate gene approach, which focuses on genes critically selected by their biological function, can be an efficient and more cost-effective strategy because SNPs that are associated with RA are mostly within genes that are functionally implicated in the pathogenicity of RA [13]. Above all, comparative genetic analysis across different ethnic groups is ideal.

In this study, we performed a trans-ethnic case–control association study of RA in Japanese and European RA patients by applying an SNP marker panel of 169 genes related to the immune system and drug metabolisms designed for genetic approaches of various immune-related diseases. A total of 1,509 SNPs were exhaustively genotyped in 238 RA cases and 184 controls of Japanese origin, as well as in 182 cases and 273 controls of German and French populations.

Materials and methods

Study populations

Sample collection and genotyping was initiated after approval from the local ethical committees. Written informed consent was obtained from all patients and control individuals after adequate explanation of the study. The disease subjects were recruited in Germany and in Japan. The 420 patients with RA consisted of 182 Germans from the Freiburg area and 238 Japanese from the Kyoto and Kobe areas. All subjects satisfied the American Rheumatism Association's revised criteria (1987) for classification of RA [14]. For population controls, 91 German subjects from the Freiburg area and 184 Japanese subjects were recruited. Additionally, 182 French Caucasian controls from the Epidemiological study on the Genetics and Environment of Asthma (EGEA) were used.

Selection of candidate genes and single nucleotide polymorphisms

A total of 169 genes on autosomes were chosen for case–control association studies of different immune-related diseases, such as autoimmune diseases, immune deficiencies, and allergies, as well as drug response in such diseases. The selection was made according to their biological function in the immune system, such as cytokines and their receptors, cell adhesion molecules, transcriptional factors, and genes involved in signal transduction and cell–cell communication (Table 1). Genes already known for their association with the diseases, in addition to those shown to be related to drug metabolism prior to 2006 when the SNP discovery was performed, were also included in the panel.

The identification of SNPs was performed by an exhaustive resequencing of exons and flanking regions of these genes, using 32 each of French and Japanese control DNAs. Additional SNPs located in the linkage disequilibrium (LD) blocks covering the 169 genes in the International HapMap Project were also included. Among approximately 10,000 SNPs, those having a minor allele frequency (MAF) greater than 0.05 were tested using the

Table 1 Classification of candidate genes by their immunological function

Signal transduction
ABCB1, ANK1, APOH, CARD4, CARD15, CBLB, CCND2, CCND2P, CCND3, CCNDBP1, CDC37, CDKN1A, CDKN2A2, EIF2AK2, EVI5L, GAB1, GAB2, GRB2, HCK, HSPCA, HSPCB, ITPKB, KIR3DL2, LAT, LCK, LCP2, MBL2, PTPN11, PTPN13, RASGRP1, SOCS3, SOS1, SYK, TANK, TGFB1, VAV1, VAV2, VAV3, XPO1, ZAP70, FTH1, NPM1
Transcription factors
AIRE, APOBEC3G, CCNT1, CCNT2, CDK7, CDK9, CD3EAP, NFAT5, NFATC1, NFATC2, NFATC3, NFATC4, STAT1, TBX1, TGFB1I1, TRIM21, TROVE2, TSC22D1, YBX1
Cell–cell communication
CD274, CD28, CD36, CD38, CD3Z, CD4, CD58, CD8A, CD8B, COMMD1, CTLA4, FCGR2A, FCGR3A, FER, ICAM2, ICAM3, ITGA1, ITGA2, ITGA4, ITGAL, ITGAV, ITGB1, ITGB4, PECAM1, SELL, TGFB, VCAM1, VIL2, VILL
Cytokines and receptors
FAS, FASLG, IFNA2, IFNAR1, IFNG, IFNGR1, IFNGR2, IL10, IL10RA, IL10RB, IL12A, IL12B, IL13, IL15, IL18, IL1A, IL1B, IL1R1, IL1RN, IL2, IL21R, IL2RA, IL2RB, IL4, IL4R, IL6, IL6R, IL6ST, IL7, IL8, L8RA, IL8RB, KIT, TNFRSF11A, TNFRSF13C, TNFRSF18, TNFRSF1A, TNFRSF1B, TNFRSF7, TNFSF15, TNFSF7
Metabolism
ALDH2, ANPEP, BCHE, CYP2C19, CYP2D6, CYP2E1, DPP4, DPYD, EPB41, FKBP4, GSTM4, GSTP1, NAT2, NOS2A, NQO1, NQO2, PLCG1, PPIA, SOD1, SOD2, SPTA1, SPTB, TPMT, TRIM5
Regulation factor of immune response
GYPA, GYPB, GYPC, OAS1, OAS2, OAS3, THY1
Genes in the *HLA* locus
HLA-DOB, PSMB8, PSMB9, TAP2, LTA, CSNK2B
Other
C1QBP: role in complement pathway (binding C1q protein)

GoldenGate technology marker panel selection program by Illumina Inc. (San Diego, CA, USA), and only markers with Illumina Design Scores (IDS) greater than 0.4 were chosen. SNPs in the same LD block with pairwise-r^2 greater than 0.8 were divided into subgroups, and the SNP which showed the highest IDS in each subgroup was selected. When there were multiple SNPs sharing the highest IDS, the one with the highest MAF was chosen. Following these selection steps, a total of 1,509 SNPs were finally chosen as tag SNP markers for the genotyping study.

Genotyping and quality control

Genomic DNAs were extracted from fresh peripheral blood mononuclear cells or from EBV-transformed lymphoblasts in accordance with protocols approved by the appropriate authority.

A panel of 1,509 SNPs was genotyped using a GoldenGate assay on an Illumina BeadArray genotyping platform according to the manufacturer's instructions. DNA samples were tracked using a laboratory information management system (LIMS), and genotypes were called using the Genotyping module of BeadStudio 2 software (Illumina Inc.). The results obtained were filtered on the basis of genotype call rates (success rates of >90% for marker, >95% for DNA sample).

Statistical analysis

Genotype distribution was evaluated by the Hardy–Weinberg equilibrium in the control group (χ^2 test), and the markers with p values less than 0.05 were excluded from the tests [15]. Allele frequency of each SNP was compared for association with RA between cases and controls in each population using the trend χ^2 test and a non-biased exact trend test [16], as well as the DerSimonian–Laird test for meta-analysis, using the combined results of two populations [17].

Expression analyses of the FCGR gene family

A gene-expression dataset in lymphoblastoid cell lines derived from 210 unrelated HapMap populations (GSE6536) was obtained from the Gene Expression Omnibus (GEO) database [18]. The correlation between the expression data of *FCGR1A*, *2A*, *2B*, *2C*, *3A*, and *3B*, and rs7551957 genotypes of 268 individuals (89 European, 44 Japanese, 45 Chinese, and 90 West African Yoruba) available from the HapMap phase 2 data, was examined using the calculation program recommended by the GEO. The association p values were obtained by the Joncheere–Terepstra method using R software or SPSS (version 18).

Results

Association analysis in Japanese and European populations

In order to identify genetic loci associated with susceptibility to RA, 1,509 SNP markers representing 169 candidate genes with an average number of 8.9 SNPs (ranging from 1 to 75) per gene, were genotyped in DNA samples of Japanese and European origins (summarized in Table 1). After quality control of the results, 1,375 and 1,330 SNPs in the Japanese and Europeans, respectively, were tested for association.

In the Japanese case–control analysis, a total of 41 SNPs tagging 26 genes showed a nominal p value <0.005, of which 13 SNPs belonging to ten genes were less than 0.001 (Supplementary Table 1). Of those 41 SNPs, rs17587 in exon4 of the *PSMB9* gene was non-synonymous (arginine to histidine), while the others were either in the gene-flanking regions (11 SNPs), 3′-untranslated region (one SNP) or in introns (28 SNPs). The association analysis using the European sample set identified a putative difference in allele frequency of 20 SNPs in 16 genes with a nominal p value <0.005, of which five SNPs corresponding to three genes showed a p value <0.001 (Supplementary Table 2). One synonymous SNP, rs2302872, was located in exon14 of the *DPP4* gene, while the others were either in the gene-flanking regions (11 SNPs) or in introns (nine SNPs). Markers that are located in the *HLA* locus showed association with RA in both populations. The strongest p value was observed for rs1894408, located adjacent to the *HLA-DOB* gene ($p = 7.5 \times 10^{-5}$) in the Japanese, and for rs1383266 near the *PSMB9* gene ($p = 0.0011$) in the Europeans (Supplementary Tables 1 and 2).

Meta-analysis using combined results of Japanese and European studies

To identify the genes/variants that are associated with RA in both Japanese and Europeans, we performed a meta-analysis combining the genotyping results of the two populations by using the DerSimonian–Laird test. In total, 22 SNPs corresponding to 15 genes showed a p value (*DLp*) less than 0.01 (Table 2). Among them, five SNPs, namely, rs6685859, rs7551957, rs2269304, rs4819522, and rs5746834, showed relatively modest association with RA in both populations (p = <0.05).

The strongest association was obtained for rs7551957 of the *FCGR2A* (low affinity Fc-gamma receptor IIa) gene, with $DLp = 8.6 \times 10^{-5}$ and OR = 1.58 with 95% confidence interval (CI) 1.25–1.99. This SNP showed a strong association with RA in both populations (trend χ^2 p = 0.0035 in the Japanese and $p = 0.0062$ in the Europeans). Three suggestive signals were obtained in the *DPYD* (dihydropyrimidine dehydrogenase) gene by meta-analysis: rs6685859 ($DLp = 1.3 \times 10^{-4}$ and OR = 1.64 with CI 1.27–2.12), rs7550959 ($DLp = 1.5 \times 10^{-4}$ and OR = 1.52 with CI 1.52–1.89), rs7531138 ($DLp = 1.7 \times 10^{-4}$ and OR = 1.51 with CI 1.22–1.88; Table 2). One SNP, rs2269310, in intron1 of the *SPTB* (erythrocytic spectrin beta) gene also showed a modest association ($DLp = 7.9 \times 10^{-4}$ and OR = 1.51 with CI 1.18–1.92) with RA.

Discussion

To our knowledge, this is the first report of a candidate gene-based trans-ethnic association analysis of RA. A panel of 169 genes that play important roles in immune response was extensively studied by SNP genotyping in Japanese and European populations. The *HLA* gene cluster is the major gene locus that contributes to RA susceptibility, and the *HLA-DRB1* gene is reported to be the strongest candidate [6]. In our study, the association between SNPs in the *HLA* region and RA was confirmed in both populations when the results were analyzed separately. However, these SNPs were not found to be significant in the meta-analysis. It is well known that the LD structure in this region is markedly different between ethnic groups. Hence, a larger sample size is required for sufficient statistical power to detect the same SNP as significant in both populations. Moreover, the panel used in this study only contains a single *HLA* gene, *HLA-DOB*, and not the others that are proven to be associated with RA.

The strongest association was obtained in the *FCGR2A* gene, which is located within the cluster of low affinity Fc-gamma receptor genes (*FCGR2A*, *FCGR3A*, *FCGR2C*, *FCGR3B*, and *FCGR2B*) on chromosome 1q22-23 [19]. Rs7551957 showed a strong association with RA in the two ethnicities ($p = 0.0035$ in the Japanese and $p = 0.0062$ in the Europeans), which was further confirmed by meta-analysis ($DLp = 8.6 \times 10^{-5}$;Table 2). In both populations, the variant allele frequency is higher in the RA cases (0.170 in the Japanese and 0.466 in the Europeans) compared with the controls (0.098 in the Japanese and 0.372 in the Europeans). Another SNP in the *FCGR2A* gene, rs1801274, showed a modest association in the meta-analysis ($DLp = 0.0021$). The difference in trend of p value between the two populations ($p = 0.098$ in the Japanese and $p = 0.011$ in the Europeans) may well be explained by the difference in the allele frequencies (0.225 in the Japanese and 0.500 in the Europeans). In line with our results, a recent meta-analysis of European genome-wide association studies confirmed the association of *FCGR2A* with RA risk ($p = 0.0004$) [20]. FCGRs are expressed on the surface of cells involved in the immune

Table 2 Single nucleotide polymorphisms associated with rheumatoid arthritis in meta-analysis using the combined results of Japanese and European populations

dbSNP ID	Chr.	Gene	Location	Nucleotide		Amino acid		Japanese freq. A2		Nominal trend *p*	European freq. A2		Nominal trend *p*	Meta-analysis	
				Ref. (A1)	Var. (A2)	Ref.	Var.	Case	Cont		Case	Cont		*DLp*	OR (95%CI)
rs6685859	1p21.3	*DPYD*	intron16	G	C			0.023	0.050	0.042	0.506	0.619	5.1×10^{-4}	1.3×10^{-4}	1.64 (1.27–2.12)
rs7550959			intron13	G	A			0.166	0.215	0.079	0.387	0.504	6.4×10^{-4}	1.5×10^{-4}	1.52 (1.22–1.89)
rs7531138			intron13	T	A			0.168	0.215	0.090	0.392	0.509	5.9×10^{-4}	1.7×10^{-4}	1.51 (1.22–1.88)
rs7551957	1q23.3	*FCGR2A*	5′-flanking	T	C			0.098	0.170	0.0035	0.372	0.466	0.0062	8.6×10^{-5}	1.58 (1.25–1.99)
rs1801274			exon4	A	G	His	Arg	0.176	0.225	0.098	0.412	0.500	0.011	0.0021	1.40 (1.13–1.74)
rs697846	1q42.12	*ITPKB*	3′-flanking	A	G			0.202	0.283	0.010	0.140	0.181	0.14	0.0042	1.44 (1.12–1.85)
rs1050567	2p15	*XPO1*	3′UTR	C	T			0.236	0.331	0.0053	0.102	0.133	0.17	0.0021	1.50 (1.15–1.94)
rs3770768	2p22.2	*EIF2AK2*	intron13	G	A			0.004	0.014	0.15	0.099	0.155	0.015	0.0082	1.72 (1.15–2.58)
rs926169	2q33.2	*CTLA4*	5′-flanking	G*	T			0.660	0.597	0.091	0.423	0.357	0.047	0.0087	1.31 (1.07–1.61)
rs2686399	3q.26.1	*BCHE*	3′-flanking	C*	G			0.844	0.819	0.36	0.732	0.648	0.0064	0.0077	1.37 (1.08–1.73)
rs6946119	7q21.12	*ABCB1*	3′-flanking	T	C			0.121	0.159	0.12	0.238	0.304	0.028	0.0076	1.39 (1.09–1.78)
rs2269310	14q23.3	*SPTB*	intron26	G	A			0.368	0.475	0.0054	0.108	0.147	0.073	7.9×10^{-4}	1.51 (1.18–1.92)
rs229670			intron1	A*	C			0.413	0.349	0.061	0.268	0.177	0.0011	0.0024	1.48 (1.14–1.91)
rs2269304			intron14	C*	A			0.118	0.075	0.049	0.213	0.153	0.018	0.0025	1.54 (1.16–2.05)
rs4787426	16p12.1	*IL4R*	3′-flanking	T	G			0.104	0.170	0.012	0.122	0.153	0.21	0.0076	1.49 (1.11–2.01)
rs4968681	17q23.3	*ICAM2*	5′-flanking	G	A			0.323	0.409	0.016	0.326	0.371	0.17	0.0082	1.32 (1.07–1.62)
rs7503550	17q23.3	*PECAM1*	intron4	G*	A			0.529	0.467	0.096	0.566	0.474	0.0072	0.0018	1.37 (1.12–1.67)
rs537188	19p13.2	*EVI5L*	intron11	G	A			0.085	0.105	0.34	0.140	0.212	0.0032	0.0045	1.52 (1.13–2.04)
rs347033	19p13.3	*VAV1*	intron4	T	C			0.144	0.227	0.0045	0.163	0.205	0.11	0.0028	1.50 (1.15–1.97)
rs2866370	20q12	*PLCG1*	intron1	G	A			0.039	0.077	0.022	0.050	0.078	0.080	0.0054	1.81 (1.19–2.75)
rs4819522	22q11.21	*TBX1*	exon9	C	T	Thr	Met	0.058	0.097	0.042	0.182	0.254	0.013	0.0014	1.58 (1.19–2.09)
rs5746834			3′-flanking	G	T			0.037	0.069	0.039	0.190	0.248	0.039	0.0049	1.52 (1.13–2.03)

SNPs with a *p* value (*DLp*) less than 0.01 according to the DerSimonian–Laird test are listed with odds ratio (OR) and 95% confidence interval (95%CI).

The risk allele is indicated by an asterisk if it is the reference allele.

Arg arginine, *His* histidine, *Met* methionine, *Thr* threonine, *Ref.* reference allele, *Var.* variant allele

system, and participate in diverse functions such as phagocytosis of immune complexes and modulation of antibody production by B cells. Various genetic polymorphisms of these receptors were reported to be associated with several autoimmune diseases [21, 22]. In particular, *FCGR2A* was shown to be associated to systemic lupus erythematosus [23, 24]. In the mouse model, *Fcgr3*-deficient hosts exhibit resistance to arthritis induced by collagen type II or anti-glucose-6-phosphate isomerase antibody [25]. In contrast, mice deficient for *Fcgr2b* lead to increased susceptibility to collagen-induced arthritis [26]. Theses findings suggest that expression of FCGRs on synovial cells may contribute to the antibody-triggered inflammation in joints [27]. In addition, we examined the association of rs7551957 with the expression level of the other *FCGR* family members according to the four population groups (European, Japanese, Chinese, and West African Yoruba), but linear regression analyses failed to reveal any significant associations (results not shown).

Variation in gene copy number is postulated to influence clinical phenotype. There have been conflicting reports regarding the association of copy number variations (CNV) in the *FCGR* locus with RA in Caucasian studies [28, 29]. In the vicinity of the *FCGR* locus there are at least three reported regions showing CNV, but rs7551957 is located more than 19-kb away from the nearest CNV region, which extends from the 3′-UTR of *FCGR2A* to the 3′-UTR of *FCGR2C* [30]. In addition, a careful examination of the rs7551957 genotype results did not reveal any indications of CNV in the observed cluster signals. Hence the association observed for rs7551957 with *FCGR2A* is unlikely to be caused by CNV of the *FCGR* locus.

Rs6685859 in intron16 of the *DPYD* gene showed $DLp = 1.3 \times 10^{-4}$, although trend p value was at a marginal level ($p = 0.042$) in the Japanese compared with the Europeans ($p = 5.1 \times 10^{-4}$). Again this may be due to the difference in allele frequencies (case versus control; 0.023 versus 0.050 in the Japanese and 0.506 versus 0.619 in the Europeans), because the risk allele is the same in both populations. The two other markers, rs7550959 and rs7531138, showed similar trends as rs6685859. DPYD is a pyrimidine catabolic enzyme, mutation of which leads to dihydropyrimidine dehydrogenase deficiency, putting cancer patients receiving 5-fluorouracil chemotherapy at an increased risk of toxicity [31]. To our knowledge, there have been no published reports on its association with RA or other autoimmune-related diseases to date.

Among the SNPs in the *SPTB* gene examined, three (rs2269310, rs229670, and rs2269304) showed significant *DLp* values. Rs2269310, which showed $DLp = 7.9 \times 10^{-4}$ and a significant trend p value of 0.0054 in the Japanese, did not show a similar level of association in the Europeans ($p = 0.073$). Again this may be due to a lower frequency of the variant allele in Europeans. SPTB acts to stabilize erythrocyte membranes, and mutations in the *SPTB* gene have been associated with spherocytosis type 2 [32], and neonatal hemolytic anemia [33]. Again, its association with RA is not known, although it has been previously reported that there was no significant immunoreactivity observed against spectrin in the sera of 50 RA patients [34].

In the current study, none of the SNPs remained significant after nominal trend p values were corrected for multiple testing using the Bonferroni method (data not shown). Therefore, detection of significant association requires replication analyses using independent sample sets. Nonetheless, we succeeded in the identification of several genetic variants as being associated with RA susceptibility across ethnicities, indicating the usefulness of a trans-ethnic comparison. Additional research is necessary to further confirm their association with the disease, and to elucidate their biological role in the pathophysiology of RA.

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Conflict of interest None.

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The human *AIRE* gene at chromosome 21q22 is a genetic determinant for the predisposition to rheumatoid arthritis in Japanese population

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Rheumatoid arthritis (RA) is a typical complex trait and the major cause of chronic inflammation worldwide. Although multiple genetic loci have been shown for their association with the onset of RA, they cover only a part of its genetic components and are largely ethnicity-specific. To identify novel genetic factors related to the predisposition and prognosis of RA in Japanese, we conducted a large-scale genome-wide association (GWA) study. We performed a GWA analysis by scanning the genome of 1247 RA cases and 1486 controls for 277 420 single nucleotide polymorphisms (SNPs), followed by replication analysis using two independent sample sets consisting of 1865 cases and 1623 controls, and 2303 cases and 3380 controls. We identified two SNPs, rs2075876 and rs760426, in intron of the autoimmune regulator *AIRE* gene at chromosome 21q22 that showed strong associations with the disease ($P = 3.6 \times 10^{-9}$ and $P = 4.4 \times 10^{-8}$, respectively). Rs1800250, in exon7 of *AIRE*, was in strong linkage disequilibrium ($r^2 = 0.94$) with rs2075876 and introduced an amino acid alteration (S278R) in the SAND domain of the AIRE protein. *In silico* analysis showed the decreased transcription of *AIRE* by the risk allele of rs2075876 compared with the alternative allele ($P = 6.8 \times 10^{-5}$). No correlation was observed between the rs2075876 genotype and quantitative traits reflecting the progression of RA. As AIRE is a key molecule which regulates the expression and presentation of self-antigens in thymic negative selection, its downregulation by genetic polymorphisms may result in the survival of auto-reactive T cells to trigger auto-inflammation in RA.

INTRODUCTION

Rheumatoid arthritis (RA) is a major cause of chronic arthritis worldwide and results in severe functional impairment and joint destruction. The impairment of joints and disability for social activity bring strong social and economic impact (1). Both environmental and genetic factors are considered to be associated with its onset and progression (2). Twin studies of

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the European populations showed that ~60% of RA onset could be attributed to genetic factors (3). In them, *HLA-DRB1* is the strongest genetic component of the disease beyond ethnicity, and is estimated to correspond to 30–50% of the genetic components in Europeans (4). Although extensive genetic analyses including hypothesis-independent genome-wide association (GWA) studies identified >20 genes in Europeans (5–13) and 7 genes in East Asians (14–19) as genetic risk loci for RA, they account for only a part of its genetic components. Moreover, trans-ethnic comparison demonstrated that their association with RA is mostly specific to a particular ethnicity and as little as three genes, namely, *CCR6*, *STAT4* and *TNFAIP3*, have shown their association in both populations. These results strongly suggest the existence of additional susceptibility loci to RA in East Asian populations (14–19). By these reasons, we have conducted a GWA study using large DNA collections of Japanese RA patients.

RESULTS

GWA analysis

We performed a large-scale genome scan using a Japanese DNA collection (collection 1) consisting of 1247 RA cases and 1486 general population controls with Illumina Infinium arrays (Supplementary Material, Table S1). After a standard procedure of quality control (see Materials and Methods), 241 523 single nucleotide polymorphisms (SNPs) were examined for their association with RA. Quantile-quantile plot to estimate population stratification resulted in a small inflation factor ($\lambda = 1.05$). The strongest association was detected for markers in the *HLA* locus with the strongest *P*-value of 2.4×10^{-38} for rs9296015. Another known genetic determinant, *PADI4*, also showed strong association (strongest $P = 1.8 \times 10^{-8}$ for rs2240335). Also, a modest association was found in the *CCR6* gene (strongest $P = 9.7 \times 10^{-4}$ for rs1556413) (16). However, there was no evidence of association for *STAT4* and the disease in our study ($P > 0.070$). There were no other loci that showed significant association ($P < 2.1 \times 10^{-7}$) after Bonferroni's correction for multiple testing.

We then took a strategy to select candidate genes/markers for further genotyping analysis based on their functional relevance in the immune system. For this purpose, we generated a list of SNP markers showing potential association with the disease (nominal $P < 0.001$), and investigated their chromosomal locations and corresponding genes in the order of association strength. Among the top 471 SNPs with *P*-value smaller than 0.001, we found two SNPs located in intron of the *AIRE* gene at chromosome 21q22, which is known as an autoimmune regulator. They were rs2075876 and rs760426 with *P*-value of 5.1×10^{-4} and 2.0×10^{-4}, respectively, and were ~6.7 kb apart from each other and in moderate linkage disequilibrium (LD) ($r^2 = 0.63$, Fig. 1). We performed genotyping of these two markers using an additional DNA collection (termed as collection 2) consisting of 1865 cases and 1623 controls. All the RA cases and 855 controls were newly genotyped with the Taqman method, and the genotypes of the other 768 controls were extracted from genome scan results of other population-based genetic studies. We successfully confirmed the association of rs2075876 ($P = 5.1 \times 10^{-4}$) in collection 2. The other marker, rs760426, showed a moderate association ($P = 0.011$) (Table 1).

We further examined whether or not the results of our study were reproducible in another Japanese RA GWA study of Biobank Japan Project recruiting 2303 cases and 3380 controls (termed as collection 3) (16). The statistical test again returned significant associations for these markers ($P = 3.6 \times 10^{-4}$ for rs2075876 and $P = 8.2 \times 10^{-4}$ for rs760426, Table 1). When the genotyping results of the three collections were pooled, the association *P*-value reached $P = 3.6 \times 10^{-9}$ for rs2075876 and $P = 4.4 \times 10^{-8}$ for rs760426 (Table 1).

We then investigated whether or not the association of the *AIRE* gene with RA was observed in Europeans. Our own genome scan results of German RA samples (I.M., M.L. and F.M., unpublished data) showed no associations for the SNP markers in the *AIRE* locus. Two large-scale GWA studies of European descents, namely, Wellcome Trust Case Control Consortium (9) and a meta-analysis of multiple GWA studies (12), did not identify *AIRE* as a risk locus, strongly suggesting its limited contribution to RA in East Asian populations.

Structure and organization of the human *AIRE* locus

LD structure of the chromosomal region containing rs2075876 and rs760426 was generated using Japanese HapMap results. As shown in Figure 1, rs2075876 and rs760426 are located in an LD block encompassing the 32 kb region between intron 5 of the *AIRE* gene and intron 12 of the liver phosphofructokinase *PFKL* gene. As the SNPs around the *PFKL* gene showed weaker association with RA ($P > 0.002$) than the two SNPs, we considered that the observed association with RA was most likely with the *AIRE* polymorphisms. However, both of these SNPs were located in intron and no other SNP markers in the genotyping arrays were mapped in this LD block and showed similar degree of association with RA. Hence, we searched for SNPs in dbSNP that were located in exons of *AIRE* and introduce functional alterations of the AIRE protein. There were five non-synonymous SNPs in the coding region of *AIRE* out of which rs1800520 in exon7 showed an allele frequency similar to that of rs2075876 (0.420). Rs1800520 introduced an amino acid alteration from serine to arginine at amino acid residue 278 (S278R). We genotyped rs1800520 in the DNA samples of all the cases ($n = 1865$) and a part of controls ($n = 855$) of collection 2 and found that rs1800520 was in strong LD with rs2075876 ($r^2 = 0.94$) and was also associated with RA ($P = 0.0071$).

AIRE polymorphism and expression

Although both rs2075876 and rs760426 are located in intron, they may have functional roles such as regulation of *AIRE* transcription. The correlation between these SNPs and transcription levels of *AIRE* was examined by using the expression profiles of 210 lymphoblastoid cells in Gene Expression Omnibus (GEO) database (20). As the result, the transcription of *AIRE* was decreased by the risk allele (A) of rs2075876 ($P = 6.8 \times 10^{-5}$, Fig. 2) but not by that of rs760426 ($P = 0.24$). Although we hypothesized the presence of a transcription factor-binding site around rs2075876, *in silico* study

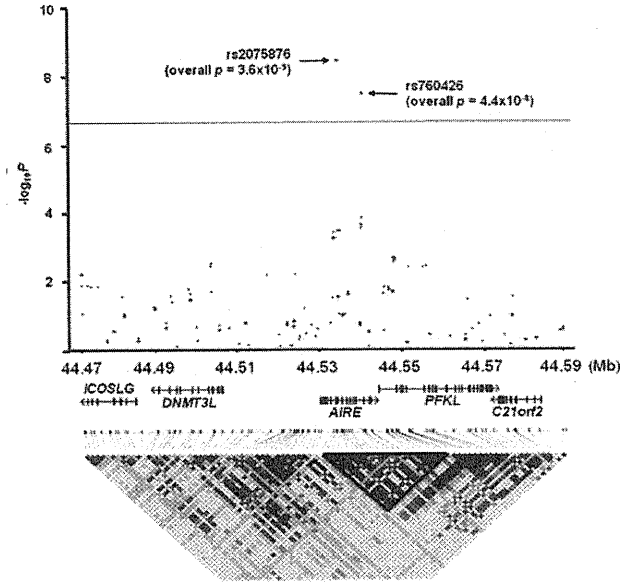

Figure 1. A schematic organization of the human *AIRE* locus at 21q22. *P*-values of the initial genome scan using collection 1 were calculated by the Trend χ^2 test and plotted in red circles. The blue circles indicate *P*-values obtained by imputation using HapMap Japanese results. Overall *P*-values of rs2075876 and rs760426 using the combined results of collections 1, 2 and 3 were also shown in green circles. A horizontal line indicates Bonferroni-adjusted $P = 2.5 \times 10^{-7}$. The structure and orientation of four genes were shown below the plots with their transcriptional orientations according to the NCBI Reference Sequence Build 36.3. LD blocks were generated according to the pairwise LD estimates of the SNPs in HapMap Japanese results.

did not predict a motif of transcription factor-binding site spanning rs2075876. Multiple nucleotide sequence alignment around rs2075876 showed a high degree of conservation among seven mammalian species (human, chimpanzee, rhesus macaque, bushbaby, horse, cow and dog). The corresponding region of rodents (mouse and rat) showed much weaker conservation (Supplementary Material, Fig. S1).

AIRE polymorphism and difference in clinical phenotypes and disease activity

RA is often subdivided into two groups based on the presence of circulating antibodies to citrullinated peptide antigen (ACPA), a specific predictive biomarker for destructive RA (21–22). In our patient collections (collection 1 and collection 2), there were 803 patients with ACPA quantification of which 176 patients were negative for ACPA. We compared the allele frequency of rs2075876 between APCA(+) and ACPA(−) groups and found no significant difference [0.39 for ACPA(+) and 0.40 for ACPA(−), $P = 0.66$]. We next tested whether rs2075876 was associated with the disease activity and prognosis. For this purpose, 212 RA patients for whom the quantitative DAS28 score was available were chosen to evaluate the correlation of RA activity and rs2075876 genotypes. Statistical analysis did not return correlations between rs2075876 genotypes and DAS28 (Supplementary Material, Fig. S2).

DISCUSSION

AIRE is a transcriptional regulator primarily expressed in medullary thymic epithelial cells (mTEC), and plays a functional role in thymocyte education and negative selection by controlling the expression of peripheral antigens in thymus (23). The expression of *AIRE* in non-thymic tissues is still controversial; some studies detected *AIRE* transcripts at a lower level in secondary lymphoid organs and in periphery while others did not (24–25), and the expression of the AIRE protein in such tissues is yet to be established. In human, dysfunction of *AIRE* caused a rare systemic multi-organ autoimmune disease known as autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED) (26). However, the patients rarely show joint destruction as observed in RA (27). In mice deficient for *aire* which develop APECED-like multi-organ autoimmune features and do not manifest with arthritis, a dramatic decrease in the expression of type II collagen was observed in mTEC and the incidence and severity of collagen-induced arthritis were augmented when compared with the wild-type (28). Such observations indicate the possible involvement of *AIRE* in immunopathology both in the human and in the mouse. However, the involvement of *AIRE* in human multigenetic autoimmune diseases still remains to be elucidated. Our study is the first successful case which clearly showed the involvement of *AIRE* in systemic autoimmunity. The function of the AIRE protein in the secondary lymphoid organs is not fully understood. Elucidation of the functions of AIRE in peripheral organs may provide hints to the involvement of *AIRE* in the predisposition or progression in RA.

In silico analysis using the GEO database showed that the risk allele of rs2075876 decreased the transcription level of *AIRE*. This may cause lower expression of various peripheral tissue antigens (PTAs), resulting in the failure of negative selection in the thymus resulting in the survival of autoreactive T cells. Although low amount of *AIRE* transcripts in B-lymphocytes was detected in most of the reported experiments, the conclusive answer for the functional impact of rs2075876 to the immune regulation needs further studies using the tissues in which *AIRE* is strongly expressed. The S278R replacement by rs1800520 is located in the SAND domain, a conserved sequence motif in nuclear proteins including Sp100 family and plays a key role in transcription regulation. However, the SAND domain of AIRE lacks the canonical KDWF motif for the interaction with DNA. Also amino acid sequence alignment of the SAND domains in different nuclear proteins revealed that S278R was located at the poorly conserved carboxyl terminal (29). Moreover, the interaction of AIRE with histone H3 through a plant homeodomain finger was suggested to be important to up-regulation of PTA genes (30). On the other hand, an assessment of mRNA stability by a computerized modeling showed lower stability of *AIRE* mRNA with the risk allele of rs1800520 than the alternative allele, suggesting the possibility of shorter half-life of the transcripts and thus lower amount of the AIRE protein. As such, we cannot conclude whether or not these SNPs have functional impact to the regulation of *AIRE* expression. The existence of unidentified SNPs that are in strong LD with them and play important functional roles is also conceivable. Extensive analyses of the *AIRE* locus by fine mapping and

Table 1. Association analysis of two SNPs in the *AIRE* gene with RA in Japanese

rs2075876		Genotype counts			Frequency A	OR (95% CI)	*P*-value
		GG	GA	AA			
Collection 1	Case	480	554	201	0.39	1.22 (1.09–1.36)	5.1×10^{-4}
	Control	639	680	167	0.34		
Collection 2	Case	706	887	243	0.37	1.18 (1.07–1.31)	9.4×10^{-4}
	Control	710	671	192	0.34		
Collection 3	Case	905	1061	330	0.37	1.15 (1.07–1.25)	3.6×10^{-4}
	Control	1462	1506	398	0.34		
Combined study	Case	2091	2502	774	0.38	1.18 (1.11–1.24)	3.6×10^{-9}
	Control	2811	2857	757	0.34		
rs760426		Genotype counts			Frequency G	OR (95% CI)	*P*-value
		AA	AG	GG			
Collection 1	Case	464	559	219	0.40	1.23 (1.10–1.38)	2.0×10^{-4}
	Control	608	709	169	0.35		
Collection 2	Case	684	897	265	0.39	1.13 (1.03–1.25)	0.011
	Control	666	741	205	0.36		
Collection 3	Case	866	1078	357	0.39	1.14 (1.06–1.23)	8.2×10^{-4}
	Control	1408	1520	450	0.36		
Combined study	Case	2014	2534	841	0.39	1.16 (1.10–1.22)	4.4×10^{-8}
	Control	2682	2970	824	0.36		

OR, odds ratio; 95% CI, 95% confidence interval.

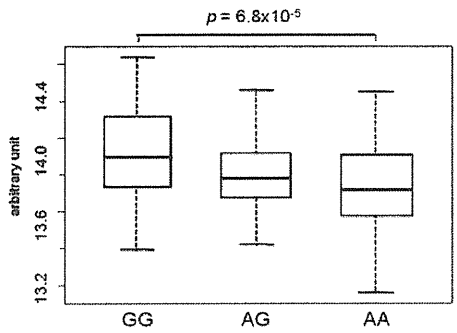

Figure 2. Comparison of the expression levels of *AIRE* among three subgroups of cell lines according to the genotype of rs2075876. 'G' and 'A' correspond, respectively, to the risk and the alternative alleles of rs2075876.

extensive sequencing in combination with examination of promoter activity will answer this question.

There was no association between *AIRE* and RA in Europeans even in the large-scale meta-analysis of GWA studies with a strong detection power (12). Although the frequency of the risk allele of rs2075876 is much lower in Caucasians (0.15 in Caucasian HapMap results and 0.097 in our own genome scan results) compared with that of the current study (0.34), this does not fully explain the lack of association in Europeans. This suggests that the association of *AIRE* with RA is, like that of *PADI4*, specific to East Asian populations including Japanese. The future validation study using other Asian population will address this issue.

MATERIALS AND METHODS

Study subjects

RA collections 1–3 consisted of 1247 affected individuals and 1486 controls, 1865 cases and 1623 controls, and 2303 cases and 3380 controls, respectively (summarized in Supplementary Material, Table S1). The case subjects of collections 1 and 2 were recruited at the rheumatology departments of Kyoto University Hospital, Dohgo Spa Hospital, Sagamihara National Hospital, Tokyo University Hospital and Tokyo Women's Medical University. The control subjects for collection 1 were from Aichi Cancer Center Hospital and Research Institute and the Department of Ophthalmology and Visual Science at Kyoto University Hospital. DNA samples of healthy Japanese volunteers in collection 2 were from Pharma SNP Consortium (31) and the Center for Genomic Medicine, Graduate School of Medicine, Kyoto University. The case and control subjects in collection 3 were recruited in the Biobank Japan Project at the Institute of Medical Science, the University of Tokyo; the Department of Allergy and Rheumatology, Graduate School of Medicine, the University of Tokyo (32). All cases fulfilled the revised criteria (1987) of the American College of Rheumatology for RA. Among the RA cases, DAS28 score for RA activity in 212 RA patients was obtained at each institution. Written informed consent was obtained from all the participants at the institute of sample collection after being approved for genetic studies by the local ethical committee.

GWA analysis

Genome scan for collection 1 was performed using Infinium Technology (Illumina Inc., San Diego, CA, USA). Case subjects were genotyped with Human-Hap300 (version 1.0, 302 627 SNPs), Human CNV370-Duo (version 1.0, 332 270 SNPs) or Human610-Quad (version 1.0, 577 348 SNPs). For control subjects, they were genotyped with Human610-Quad (version 1.0, 577 348 SNPs) and HumanHap550 (version 3.0, 547 163 SNPs). For validation analysis, Taqman

technology (Life Technologies Corp., Foster City, CA, USA) was employed.

Quality control and statistical tests for case–control association

A total of 277 420 SNPs that were common among the four types of arrays described above were selected for the association study. One thousand two hundred and forty-six cases and 1486 controls with call rate being >0.90 and not showing high degree of kinship (PI_HAT < 0.10 by PLINK) were examined for the association analysis. A total of 241 523 SNPs with call rate >0.95 for both cases and controls and minor allele frequency >0.05 either in the case or in the control were used for the analysis. The case–control association was examined with the Cochran–Armitage trend for each collection as well as for the combined pooled study. Population stratification was examined and corrected with Genomic Control. SNPs that showed *P*-value $<10^{-3}$ were selected as candidates for further evaluation. SNPs in the *HLA*, *PADI4* and *CCR6* loci were not selected for validation studies. Haploview version 4.1 software (33) was used for LD evaluation, and MapViewer (build 36.3) (34) was used to identify the location and structure of the genes in the region.

Analysis of *AIRE* expression

A gene-expression data set in lymphoblastoid cell lines derived from 210 unrelated HapMap populations was obtained from GEO database (20). The correlation between the expression of *AIRE* and genotypes of SNPs in the region was examined using the calculation program recommended by GEO. The association *P*-values were obtained by the Joncheere–Terepstra method using R software or SPSS (version 18).

Bioinformatics analysis

Genome sequence alignment of 14 placental mammals was obtained from the UCSC genome browser (http://genome.ucsc.edu). Motif search was carried out by the Jaspar database (35) (http://jaspar.cgb.ki.se) using 'Jasper Core Subset' which contains 138 matrices for known *cis*-acting elements. The matrices were converted into bit scores and used to search against the genomic sequences around the SNP of interest. Identification of orthologs of the *AIRE* gene in different mammals and multiple nucleotide sequence alignment was performed using KEGG SSDB Database (www.genome.jp/kegg/ssdb).

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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Conflict of Interest statement. None declared.

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APPENDIX

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PLoS one

Association between the SERPING1 Gene and Age-Related Macular Degeneration and Polypoidal Choroidal Vasculopathy in Japanese

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Abstract

Purpose: Recently, a complement component 1 inhibitor (*SERPING1*) gene polymorphism was identified as a novel risk factor for age-related macular degeneration (AMD) in Caucasians. We aimed to investigate whether variations in *SERPING1* are associated with typical AMD or with polypoidal choroidal vasculopathy (PCV) in a Japanese population.

Methods: We performed a case-control study in a group of Japanese patients with typical AMD (n = 401) or PCV (n = 510) and in 2 independent control groups—336 cataract patients without age-related maculopathy and 1,194 healthy Japanese individuals. Differences in the observed genotypic distribution between the case and control groups were tested using chi-square test for trend. Age and gender were adjusted using logistic regression analysis.

Results: We targeted rs2511989 as the haplotype-tagging single nucleotide polymorphism (SNP) for the *SERPING1* gene, which was reported to be associated with the risk of AMD in Caucasians. Although we compared the genotypic distributions of rs2511989 in typical AMD and PCV patients against 2 independent control groups (cataract patients and healthy Japanese individuals), *SERPING1* rs2511989 was not significantly associated with typical AMD (P = 0.932 and 0.513, respectively) or PCV (P = 0.505 and 0.141, respectively). After correction for age and gender differences based on a logistic regression model, the difference in genotypic distributions remained insignificant (P>0.05). Our sample size had a statistical power of more than 90% to detect an association of a risk allele with an odds ratio reported in the original studies for rs2511989 for developing AMD.

Conclusions: In the present study, we could not replicate the reported association between *SERPING1* and either neovascular AMD or PCV in a Japanese population; thus, the results suggest that *SERPING1* does not play a significant role in the risk of developing AMD or PCV in Japanese.

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Introduction

Age-related macular degeneration (AMD) is the leading cause of visual loss in the developed world [1]. Several genes have been reported to be associated with this disease, including complement factor H [2–4] and the age-related maculopathy susceptibility 2/HtrA serine peptidase 1 (ARMS2/HTRA1) region [5,6], and subsequent studies have replicated the association between susceptibility genes and the development of AMD using a different ethnic cohort [7–10].

Inner choroidal vascular networks that terminate in polypoidal lesions are defined as polypoidal choroidal vasculopathy (PCV), and are typically visualized by indocyanine green angiography [11]. Whether PCV represents a subtype of neovascular AMD remains controversial; moreover, whether this condition represents inner choroidal vascular abnormalities or is a variety of choroidal neovascularization remains unknown [12]. Previous studies identified several genes that contribute to the development of PCV; however, almost all reported genetic risk factors for PCV are the same as for AMD [13–15], and this suggests that AMD and PCV share, at least in part, the same genetic background.

Studies in cohorts from both the United Kingdom and the United States have shown that the complement component 1 inhibitor (*SERPING1*) gene is positively associated with AMD [16]. However,

Table 1. Characteristics of the Study Population.

		Cases		Controls	
		tAMD	PCV	Control 1*	Control 2†
No. of participants		401	510	336	1194
Age	Mean ± SD	77.38±8.39	74.98±7.77	74.16±8.42	50.34±15.9
Gender	Men	287	372	142	493
	Women	114	138	194	701

tAMD, typical age-related macular degeneration; PCV, polypoidal choroidal vasculopathy; SD, standard deviation.
*Cataract patients without age-related maculopathy.
†Healthy Japanese individuals.
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another study in a larger cohort (n = 7723 and 2327) which involved the same population could not replicate the finding of the previous study [17,18]. Recently, Lee et al. have shown that *SERPING1* is positively associated with AMD in Caucasians [19], but whether this gene is truly associated with AMD remains controversial.

Furthermore, the association of *SERPING1* with AMD has been evaluated also in Asians. Lu et al. examined the association in 194 AMD patients and 285 controls and reported that *SERPING1* is not associated with AMD in the Chinese population [20]. The association between PCV and *SERPING1* has also been evaluated in a smaller Chinese cohort (118 patients and 115 controls), also with negative findings [21]. So far, all Asian studies for *SERPING1* did use smaller cohorts than those of original studies and not consider their statistical power. For evaluating the true gene-disease association, it would be helpful to replicate the positive association reported in previous studies using a different ethnic cohort. The aim of this study, which involved a relatively large number of participants, was to investigate whether the *SERPING1* gene variants are associated with typical AMD or PCV in a Japanese population.

Materials and Methods

All procedures in this study adhered to the tenets of the Declaration of Helsinki. This study was approved by the Ethics Committee of each institute involved (Kyoto University Graduate School and Faculty of Medicine, Ethics Committee, the Ethical

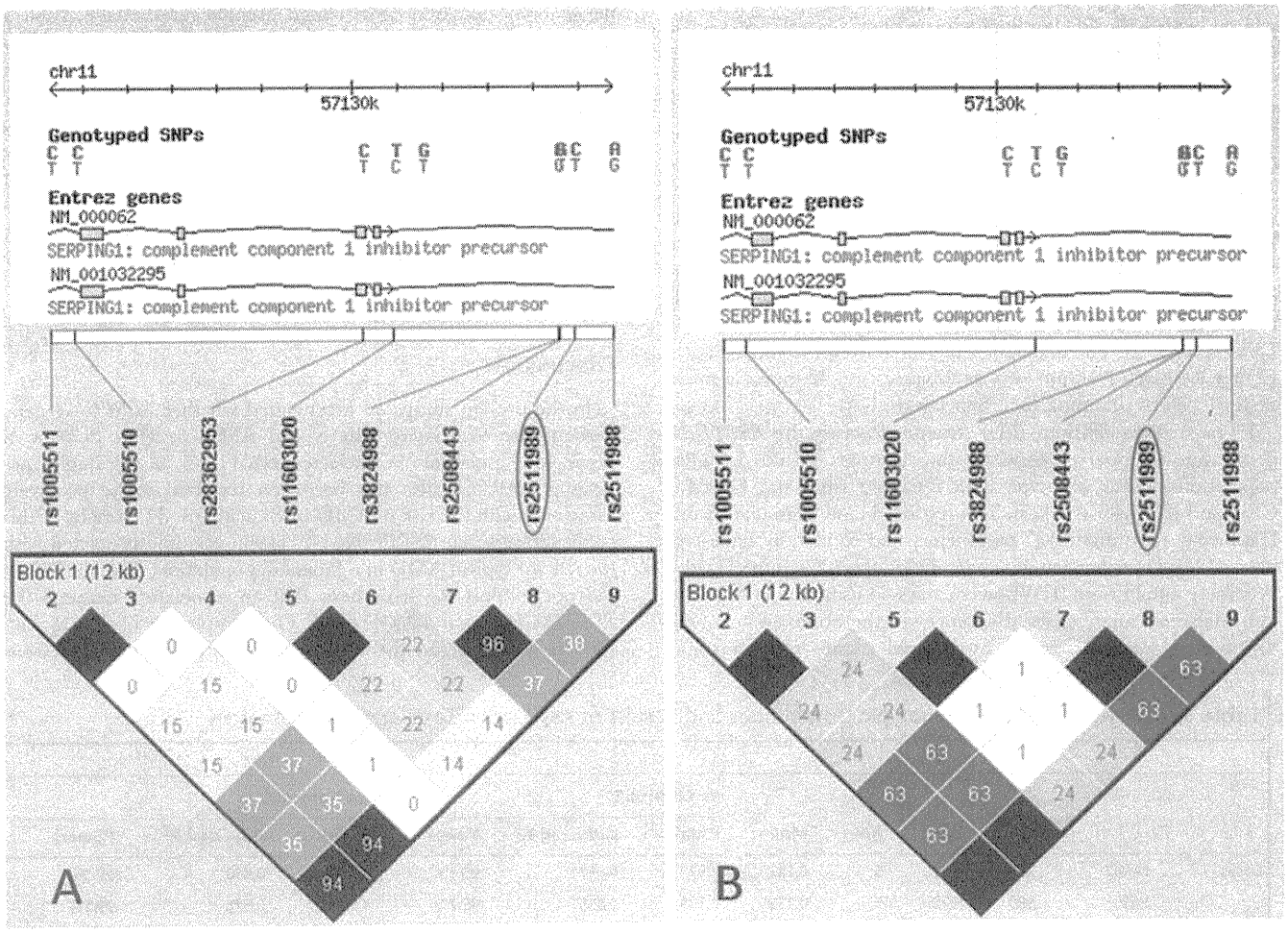

Figure 1. Linkage disequilibrium (LD) structure across the complement component 1 inhibitor (*SERPING1*) gene in Caucasian and Japanese populations. Genotype data were retrieved from HapMap CEU (Utah residents with ancestry from northern and western Europe; A) and JPT (Japanese in Tokyo, Japan; B) data sets. Haplotype blocks were determined using the "four-gamete rule" option in Haploview; all HapMap single nucleotide polymorphisms on *SERPING1* gene are in the same block in both populations. Each box provides estimated statistics of the coefficient of determination (r^2), with darker shades representing stronger LD.
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