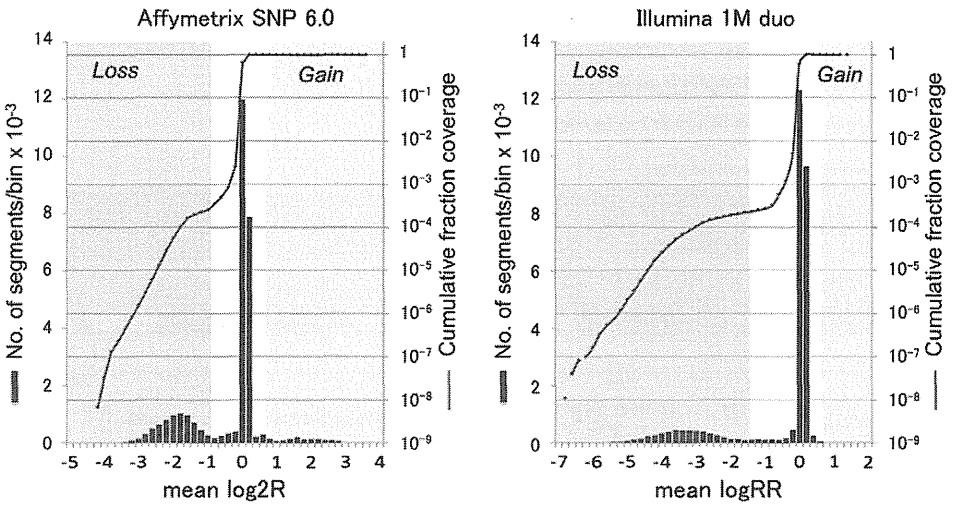


Fig. 3. Distribution of copy number segments in bins of mean relative signal intensities. See text for detail.

was, forcing all calls at log2R < −0.6 (0.88% of *Affymetrix* calls), or logRR < −1 (0.17% of *Illumina* calls) to no-calls. We also removed 164 SNPs in *Illumina* calls, because they were duplicated (i.e., two SNP at the same position). Subsequently, SNPs with call rate less than 90% were removed. After these quality control steps, 84 CHMs, whose SNP genotypes were called at greater than 96% by both platforms, remained.

The genotypes of both platforms were compared using merge function of *PLINK* program version 1.07 [2], that revealed considerable strand inconsistencies between the two platforms. We flipped the strands of *Illumina* data for these SNPs to resolve inconsistency with *Affymetrix* annotation. After these corrections, the fraction of discordant calls was 1.05×10^{-5}, which were forced to no calls at merge (Fig. 2).

Table 1
CNV segments defined by the two platforms.

Platform	Loss (per genome)	Gain (per genome)
Affymetrix SNP 6.0	6517 (78)	1444 (17)
Illumina 1 M-duo	4597 (55)	39 (0.5)

The definition of gain CNV segments is arbitrary. See text for detail.

Linkage disequilibrium, LD bins and tagSNPs

The pair-wise r^2 values between merged SNP markers whose minor allele frequencies were at least 5% (common SNPs) and maximum inter-marker distance of 300 kb were calculated. LD bins were determined at threshold of $r^2 \geq 0.80$ by *TagZilla* version 1.0 (http://tagzilla.nci.nih.gov/). The program estimates LD bins using a greedy maximal approach similar to that of *ldSelect* [3]. As a result, 1,115,537 common SNPs were grouped in 366,214 LD bins, of which 189,417 were single-SNP bins. That left 17% of common SNPs without proxies. TagSNPs (representative SNPs for each bin) was selected by the *TagZilla* criteria "avesnp", that is, having maximum average r^2 with all other SNPs in the bin.

CNV segments and CNV regions

B allele frequency (BAF) of heterozygous sites has been commonly used as an indicator of CNV of *Illumina* array data obtained from diploid materials. However, it is not an appropriate indicator in this study, because all SNPs in our duplicated haploid samples are expected to be genome-widely homozygous. And so, relative signal intensity of

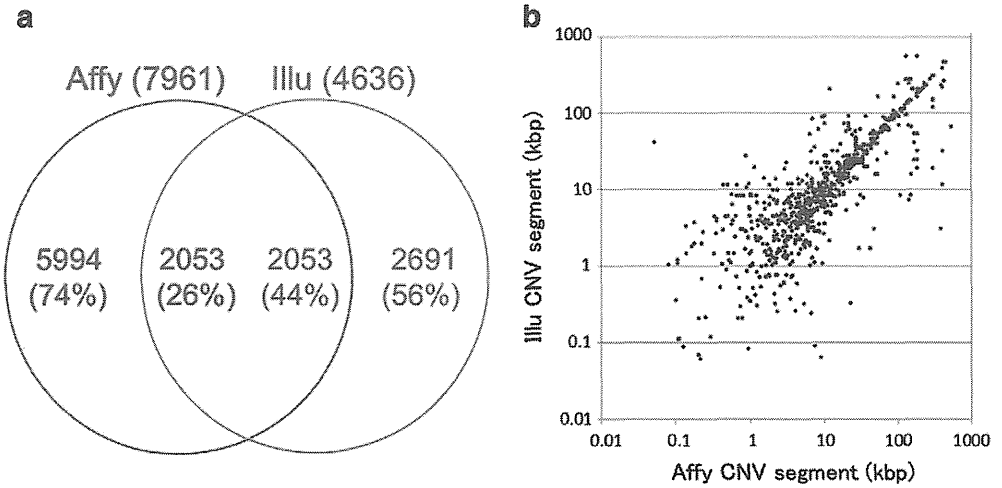
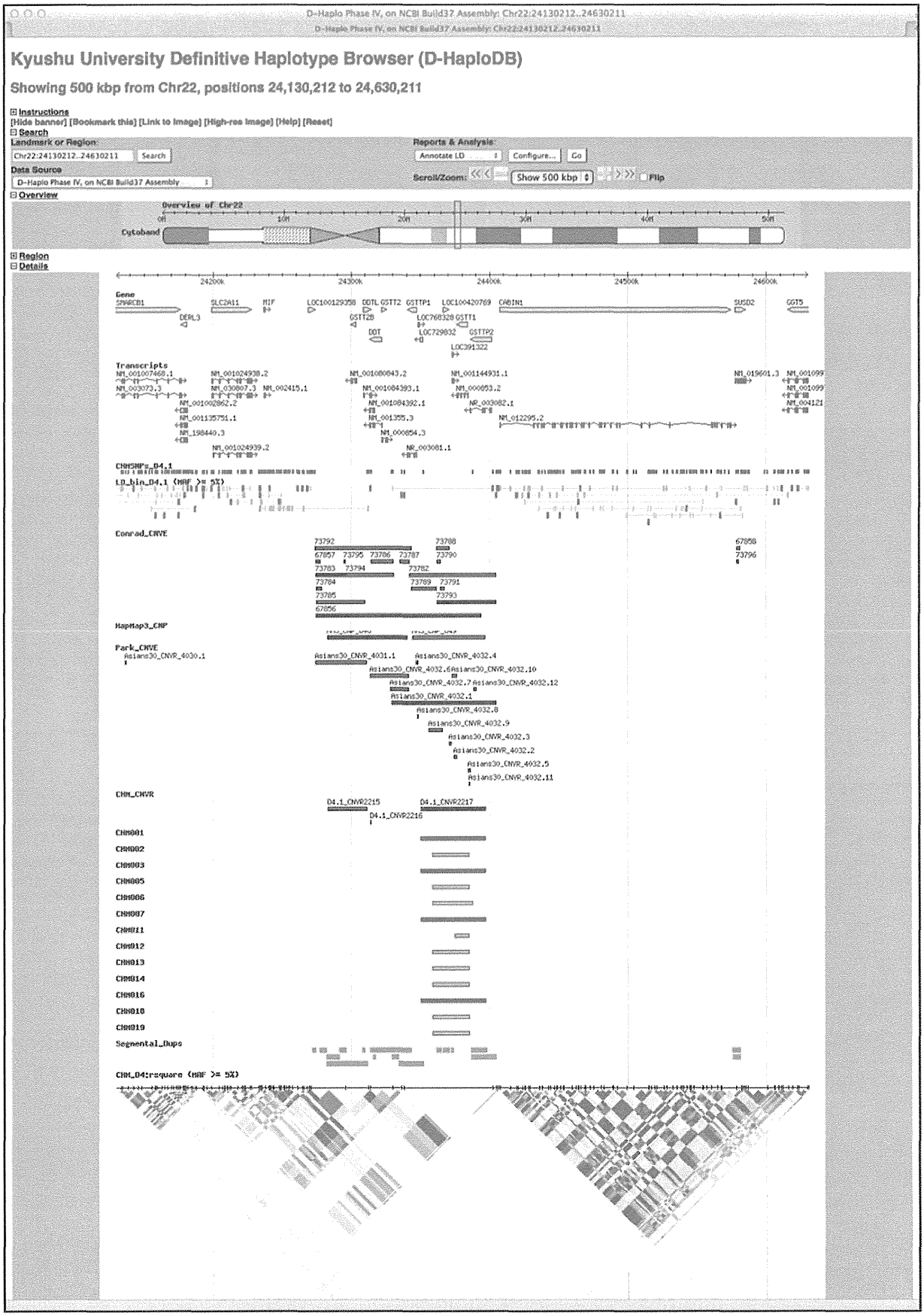


Fig. 4. Overlap and size correlation of CNV segments detected by two platforms. a. The concordant calls of CNV segments between *Affymetrix* system and *Illumina* system were examined without distinguishing gains or losses, as detailed in the text. b. The lengths of overlapped CNV segments detected in the *Affymetrix* (abscissa) and *Illumina* (ordinate) systems are plotted.


D-Haplo Phase IV, on NCBI Build37 Assembly: Chr22:24130212..24630211
Kyushu University Definitive Haplotype Browser (D-HaploDB)
Showing 500 kbp from Chr22, positions 24,130,212 to 24,630,211
Instructions
[Hide banner] [Bookmark this] [Link to image] [High-res image] [Help] [Reset]
Search
Landmark or Region:
Chr22:24130212..24630211
Search
Data Source
D-Haplo Phase IV, on NCBI Build37 Assembly
Reports & Analysis
Annotate LD
Configure...
Go
Scroll/Zoom:
Show 500 kbp
Flip
Overview
Overview of Chr22
Cytoband
Region
Details
Gene
Transcripts
CHMSNPs_D4.1
LD_bin_D4.1 (MAF >= 5%)
Conrad_CNVR
HapMap3_CNP
Park_CNVR
CHM_CNVR
CHM001
CHM002
CHM003
CHM005
CHM006
CHM007
CHM011
CHM012
CHM013
CHM014
CHM016
CHM018
CHM019
Segmental_Dups
CHM_D4:rsquare (MAF >= 5%)

markers is the only variable for the detection of copy number changes, which we detected using circular binary segmentation algorithm implemented in the *R* statistical package module *DNAcopy 1.26* with default parameters [4]. Since the distributions of log2R and logRR were widely different, combined interpretation of the two data sets were inappropriate. Therefore, the segmentation analysis of the two data sets was carried out separately.

Fig. 3 shows the distribution of mean relative signal intensities of segments defined by the two data sets (*Affymetrix* and *Illumina*). As shown in the figure, distinct peaks were observed in the regions below zero, apparently distinguishing deletion segments from normal copy segments. We defined the boundary of the two copy number states at the inflection points of cumulative segment coverage in each data set. Thus, the copy number states of segments having mean log2R < -1 for *Affymetrix* and mean logRR < -2 for *Illumina* were defined to be a loss, that accounted 0.02–0.03% of the genome. The thresholds for the definition of gain segments were not distinguishable from the plots, and we arbitrarily placed the boundary at 0.5 for both data sets. Then, a CNV segment that extended beyond centromere was split at the latter. The segments were filtered so that all of them had the sizes greater than 50 bp. The numbers of CNV segments defined by the two platforms are summarized in Table 1.

The concordance of CNV segment calls between the two platforms was examined using an "intersect" function of *BEDTools* version 2.11.2 [5], setting a minimal overlap of one bp. The results revealed that in some genomic regions, mutually exclusive subsets of samples were judged to be in the CNV segments of opposite directions (gain by *Affymetrix* versus loss by *Illumina*). We also found that less than half of segments detected by the two arrays were overlapped (Fig. 4a). The reason for these apparent discrepancies should at least partly be attributable to the differences in the definition of reference intensities in the calculation of relative signal intensity and in the distribution of markers between the two systems, as discussed previously [1]. However, a good size correlation between overlapped segments was observed for segments longer than 10 kb, although some discrepancies by splitting/fusion of overlapped regions between the two platforms were observed even in long segments (Fig. 4b).

Next we defined CNV regions as merges of CNV segments across CHM samples without discriminating gains or losses. The results revealed a total of 2339 CNV regions that occupied 1.4% of the genome.

Definitive Haplotype Database (D-HaploDB)

The results of SNP genotypings and CNV analyses described above are comprehensively presented in tracks (listed below) of *D-HaploDB* version 4.1 (http://orca.gen.kyushu-u.ac.jp) that uses *Generic Genome Browser* version 1.64 [6]. The genome coordinates are according to *GRCh37*. A screen shot of an example page of the database is shown in Fig. 5.

- CHMSNPs_D4.1: Merged SNPs genotyped using *Affymetrix* and *Illumina* platforms, and validated. Individual genotypes and allele counts are viewable by clicking the glyphs.
- Affymetrix SNP 6.0: Positions of *Affymetrix* markers are shown, with distinction of SNP probes (red) and CN probes (black).
- Illumina 1 M-duo: Positions of *Illumina* markers are shown, with distinction of SNP probes (red) and intensity only probes (black).
- LD_bin_D4.1 (MAF $\geq$ 5%): The pair-wise r^2 tagging at $r^2 \geq 0.8$ using *Tagzilla 1.0* program was done for SNPs whose minor allele frequencies were at least 5%. The best-tags (i.e., the tagSNP that showed the highest average r^2 against the remaining members within the bin) are highlighted in red. Details containing SNP and haplotype information are viewable by clicking the glyphs.
- r-square (MAF $\geq$ 5%): The r^2 values from high to low between all combinations of markers within the selected regions are graphically shown by deep to shallow red.
- CHM_CNVR: CNV regions (CNVRs) in CHMs were defined as merges of CNV segments across all CHM samples. Thus, these are the regions where CNV segments were detected by either *Affymetrix* or *Illumina* platforms at least in one CHM.
- CHM#: CNV segments in each CHM sample (indicated by #) are shown with distinctions of losses (red) or gains (blue), and *Affymetrix* (dark) or *Illumina* (light).

In addition, some external data are incorporated and presented in tracks, to facilitate further interpretation of our data. Those are cytobands, genes, transcripts, segmental duplications and CNV data of Conrad et al. [7], HapMap3 [8], and Park et al. [9].

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Fig. 5. Screen capture of D-Haplo D4.1 glutathione S-transferase theta 1 region. CNV segments of gain or loss was detected by Affymetrix or Illumina systems, respectively, for mutually exclusive subsets of CHM samples. CNV segments of only a portion of samples are shown for the ease of viewing.

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INVITED REVIEW

Recent advances in Takayasu arteritis

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Abstract

Takayasu arteritis (TAK) is a relatively rare systemic vasculitis mainly affecting the aorta and its large branches. While patients with TAK are more frequently observed in Asian countries, we can find patients with TAK all over the world. This limited number of patients has made it difficult to collect large numbers of patients and perform detailed studies. However, recent progresses have led to the identification of susceptibility genes and novel susceptibility human leukocyte antigen (HLA) alleles as well as accumulation of clues for the pathophysiology of TAK. *IL12B* was shown to be a susceptibility gene beyond ethnicity. *MLX* and *FCGR2A/3A* were shown to be associated with TAK in Japanese and Turkish/American populations, respectively. HLA-B*52:01 and *67:01 are susceptibility alleles to TAK, and the 171st and 67th amino acid residues of HLA-B protein are suggested important for TAK susceptibility. *HLA-DQB1/DRB1* is recently reported as an independent susceptibility locus. Although there are no standardized serum markers or composite measures for disease activity of TAK, Japanese and Italian groups showed pentraxin 3 as a novel biomarker for detecting and monitoring patients with TAK. Recently, an Indian group proposed a novel scoring system called ITAS to evaluate disease activity of TAK. Standardization of assessing disease activity would lead to clinical studies with high quality. Several groups reported results of treatment for refractory TAK with biological agents targeting tumor necrosis factor or interleukin-6R. The recent accumulation of research data should improve understanding of the basic pathophysiology of TAK and lead to better management of patients with TAK.

Key words: clinical aspects, disease aetiology and pathogenesis – human, drug treatment, epidemiology, genetics.

INTRODUCTION

Takayasu arteritis (TAK) is a systemic vasculitis mainly affecting the aorta and its large branches.[1] TAK was first reported by Mikito Takayasu, a professor of ophthalmology at Kanazawa University, Japan in 1908.[2] The first case was a 21-year-old woman complaining of lowering vision. This episode told us that patients with this disease present with a wide range of symptoms. TAK is classified as one of the two arterites affecting the large arteries.[3] The other is giant cell arteritis (GCA), which was previously called 'temporal arteritis'. In this manuscript, we review the latest study results as well as previous literatures and revisit the basics of TAK.

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CLINICAL ASPECTS

Prevalence

Although a relatively large number of patients with TAK are observed in Asian countries, patients with TAK have been reported from all over the world.[4] However, previous studies addressing the prevalence of TAK are quite limited. In Japan, a total of 56 diseases, including TAK, are defined as intractable diseases and patients are subjected to a nation-wide questionnaire about their clinical status and history, which is filled in by the clinicians providing their care.[5] According to this nation-wide registry, there were at least 5881 TAK patients in Japan in 2012. Because the primary motive of this registry of

clinicians and patients should be financial support for care in TAK, patients with TAK whose disease activity is stable might be missed in this registry. Thus, the real number of patients with this disease should be larger than 6000 in Japan. Considering the population in Japan, the prevalence is more than 0.004%.

Clinical manifestations

Clinical manifestations include fever, fatigue, weight loss, headache, faintness, difference of arterial pressure between bilateral upper or lower limbs and symptoms from severe complications. Long inflammation in branches of the aorta leads to narrowing and occlusion of these arteries and branches. In severe cases, it is very hard to feel pulses in patients with TAK. This is why TAK is also called 'pulseless disease'. Complications include aortic regurgitation (AR), pulmonary thrombosis, cerebral infarction, hearing problems, lowering of vision, and in worst cases, blindness. Although the life expectancy of patients with this disease was estimated to be low, the introduction of glucocorticosteroids and immunosuppressants has dramatically improved prognosis of this disease. In fact, prognosis is reported to have improved in patients diagnosed after 1976 compared with patients diagnosed before 1975.[6] This improvement may be partly explained by the development of treatment for this disease and the wide understanding of this disease across physicians. However, this also suggests that the natural course of this disease has been improved by unknown reason(s).

Classifications of disease types

Hata *et al.* reported classification of this disease based on distribution of aortic lesions.[7] However, there are no studies to date supporting associations between these subtypes and clinical outcome and markers. A recent study has suggested that expression of Toll-like receptor patterns are associated with distribution of arterial lesions.[8] Another recent study from France suggests common patterns of involvement of arterial branches in patients with TAK.[9] As we discussed above, GCA is the other vasculitis affecting large arteries. Recently, the similarities between TAK and GCA have been drawing attention.[10] Although both diseases clearly have a different etiology, there are many common pathological findings. GCA mainly affects older populations. Giant cells and granulomatous lesions can be found in patients with TAK and GCA. Maksimowicz-McKinnon *et al.* analyzed 69 and 75 patients with GCA and TAK, respectively, and found 73% of patients with GCA have lesions in large branches of the aorta.[11]

Diagnosis

The criteria for TAK by the American College of Rheumatology[12] are widely used. In Japan, the guideline provided by the Japanese Circulation Society[13] is also used for diagnosis. There are no studies to date comparing the diagnostic accuracy between the different criteria, but considering the difference between the items contained in each, using one criteria does not seem to result in a big difference in accuracy compared with using the other. It has been shown that many patients were diagnosed as having TAK more than several years or as long as decades after they developed the disease.[6] A recent study reported that this discordance of time between development and diagnosis of TAK has become shorter and shorter.[14] This may reflect the development of imaging techniques and prevailing information about this disease among physicians. Because occlusion or narrowing of arteries and branches of the aorta appear in advanced stages of the disease, establishment of classification criteria, which could diagnose TAK in the early stage, is strongly desirable.

Imaging

Imaging of arteries is very useful in diagnosing TAK and for patient follow-up. Angiography is the gold standard to show narrowing or occlusion of the aorta or its main branches. Computed tomography (CT) angiography or magnetic resonance (MR) angiography are very useful tools to detect arterial lesions. Positron emission tomography (PET) is also useful to detect inflammation of arteries.[15] Atherosclerosis may display similar signals in PET so that special attention needs to be paid to aging and basic metabolic disease status to accurately evaluate the results of PET. Since establishment of classification criteria for early TAK is desired, PET could serve to detect active disease lesions before occlusion or narrowing of large branches of the aorta. Incorporation of MRI with enhancement or FDG-PET (PET with ^{18}F-fluorodeoxyglucose) would improve accuracy of early diagnosis.[15,16]

Biological markers

To date, no established biological markers specific to diagnose patients with TAK have been reported. Patients with TAK often present with increased inflammation markers, including C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). As for biological markers to follow patients with TAK, reflecting the daily activity of TAK, inflammation-related markers

are often used in daily medical care. Although CRP and ESR are often useful to follow patients with TAK, some patients suffer from worsening of vasculitis without increasing CRP or ESR. Thus, biological markers which surpass CRP or ESR or function as compensation of these markers are required. A Japanese group reported matrix metalloproteinase (MMP)-2, -3 and -9 as useful to assess disease activity and follow TAK patients.[18] Since an increased level of MMP-3 according to prednisolone usage[17] has been reported, MMP-3 levels should be carefully interpreted. Serum levels of interleukin (IL)-6, regulated upon activation, normal T expressed and secreted (RANTES), vascular cell adhesion molecules (VCAM) are also increased in patients with TAK.[18–21] IL-6 is also reported to be associated with TAK disease activity. IL-6 activates B cells and T cell cytotoxicity and promotes production of inflammatory cytokines. Recently, two teams from Japan and Italy identified pentraxin 3 (PTX-3) as a promising serum marker for TAK to follow its activity.[22,23] The Italian team reported that PTX-3 provided better area under curve in receiver operating curves to detect active patients with TAK. The Japanese group reported six out of eight patients presented increased levels of PTX-3 without any increase in CRP levels. PTX-3 might serve as a marker to follow patients who develop progressive occlusion of the aorta in spite of negative CRP cases.

Composite measure

Disease Extent Index in Takayasu arteritis (DEI.Tak) is a novel measurement without imaging to follow-up patients with TAK and is reported to be useful to assess disease activity and extent of damage from TAK.[24] Recently, the Indian Takayasu arteritis consortium proposed Inidian Takayasu Clinical Activity Score (ITAS2010), a novel method of evaluating TAK disease activity.[25] They also expanded ITAS2010 to ITAS2010-A by incorporating acute-phase reactants.[25] This Indian study is the largest study following patients with TAK and assessing disease activity. Standardization of composite measures to assess disease activity in TAK would make clinical examinations easier in a multi-ethnic manner. It should be noted that there is no evidence concerning the usefulness of the novel markers and composite measures for improving prophylaxis of patients with TAK. A large-scale, consecutive, longitudinal study would elucidate the applicability of the markers and measures. To achieve the final goal of freedom from vascular damage, we should clarify targets in daily medical care.

Treatments

Glucocorticosteroids are anchor drugs for this disease, like other vasculites. Most cases in Japan respond with 0.3–0.5 mg/kg/day predonisolone, but we frequently found that some patients present with flare-ups during tapering of glucocorticosteroids. Since TAK mainly affects young women, side-effects of glucocorticosteroids, especially moon face, severely damage their quality of life. Immunosuppressive agents, including methotrexate, cyclosporine, cyclophosphamide, mycophenolate mofetil and taclolimus have been used for patients with TAK. Biological agents targeting tumor necrosis factor (TNF) have also been used for patients with TAK. Cliffold *et al.* recently reviewed literature on patients with TAK treated by anti-TNF agents.[26] While there are more than five biological agents which target TNF, the majority of the 120 patients with TAK treated with anti-TNF agents received infliximab. They found that approximately 90% of the patients responded to anti-TNF agents, but at the same time, they reported that about 40% of these patients relapsed. Since patients treated with anti-TNF agents other than infliximab are limited, it is hard to detect differences in efficacy among different anti-TNF agents. Tocilizumab (TCZ), humanized anti-IL-6R antibody, has also been recently used for patients with TAK.[27] Although each study has a limited number of patients, Japanese, Italian and UK groups reported favorable effects and good tolerance of TCZ in patients with TAK. Abisror *et al.* recently reviewed literature on patients with TAK treated with TCZ[28] and they found that a total of 44 patients with refractory TAK showed 75% efficacy of TCZ at their last visit. It should be noted that long-term outcome in patients with TAK treated by these biological agents was not assessed in these studies. We should also pay attention to publication bias, but these favorable results might indicate efficacy of biological agents for TAK. Importantly, physicians successfully decreased the amount of oral glucocorticosteroids in most cases treated with biological agents. In some cases, they can cease oral glucocorticosteroids in patients suffering from side effects. Thus, double-blind randomized case control trials (RCT) or large-scale open label studies would be very interesting. RCT for abatacept, CTLA4-Ig, is now recruiting patients with TAK and GCA in US. Considering the number of patients with TAK, the development of a novel biological agent for this disease would be extremely difficult. However, when we find that treatments currently available for other diseases are also effective for this disease, such repurposing of the drugs

would bring a lot of promising options in patients with TAK.

The animal model of aortitis in IL-1 receptor antagonist (IL-1Ra)-deficient mice raised the possibility of a therapeutic use of IL-1 blockade therapy in patients with TAK.[29] However, there is no report of using anakinra, a representative IL-1 blockade, for patients with TAK, in spite of case reports of successful treatment in patients with GCA.[30] Furthermore, due to the short half-life of this drug, long-term usage of anakinra might be a problem in terms of frequent injection in patients with TAK unless anakinra shows marked efficacy compared with other biological agents. Ustekinumab, a monoclonal antibody against IL12p40, is an efficient therapeutic option for psoriasis and Crohn's disease,[31,32] in both of which the *IL12B* region is associated. As we discuss later, the *IL12B* region is also associated with TAK. These data strongly suggest that ustekinumab would be a very promising therapeutic option for patients with TAK.

BASICS

Genetics

The only established genetic component associated with TAK has been HLA-B52. The association between HLA-B*52:01 and TAK has been repeatedly shown in different populations.[33–38] There are studies reporting the importance of other alleles, including HLA-DPB1 or HLA-DRB1 alleles.[39,40] The recent genome-wide association study (GWAS) showed an independent association in the *HLA-DQB1/DRB1* locus.[41] Although HLA-B51, a strong susceptibility allele to Behçet disease,[42] shares large parts of amino acid sequencing with HLA-B*52:01, the association between HLA-B51 and TAK was negatively reported.[34] Our recent work might provide an answer to these observed different susceptibilities.[38] Our study indicates the importance of the 67th and 171st amino acid residues for TAK susceptibility where the 67th is one of the two amino acid residues not shared between HLA-B*51:01 and *52:01. Furthermore, both the amino acid positions are located at peptide binding grooves,[43–45] suggesting that peptide binding at these positions would be very important for the predisposition of the two different autoimmune diseases. A previous Mexican study suggested the involvement of the 63rd and 67th amino acids.[46] Thus, different studies suggest the importance of the 67th amino acid of the HLA-B protein. Although HLA-B39 was reported to be associated with severe complications of TAK as well as TAK onset in a previous study,[47] the association was not observed in a recent Japanese study, and it did not find a different association between HLA-B67:01 and TAK clinical manifestations.[48] Our recent work confirmed this lack of association of HLA-B39 and the positive association of HLA-B*67:01.[38] HLA-B*67:01 has not been reported in Turkey and Middle-East Asia. Although GCA shows association with HLA-DR4,[49] which is not a TAK susceptibility allele, meta-analysis of TAK and GCA would reveal similarity and differences between the two large vessel arterites.

Recently, we reported the first GWAS results for this disease at the same time as a US/Turkish group.[41,50] Both groups reported *IL12B* as a strong susceptibility locus to TAK. Our group also reported the *MLX* region in chromosome 17 and a US/Turkish group reported the *FCGR2A/3A* region as another susceptibility locus. The US/Turkish group also reported the *PSMG1* region as a suggestive locus. Our data also showed that the polymorphism in the *IL12B* region is associated with high incidence of AR, severity of AR and higher time-averaged CRP level as a representative of disease activity. Furthermore, our data indicated that the polymorphism of the *IL12B* region displayed a synergistic effect on TAK susceptibility in combination with HLA-B*52:01. These results indicate that *IL12B* plays a central role in not only the development of TAK but also progression of the disease. *IL12B* encodes the IL12/23p40 protein, a common subunit of IL-12 and IL-23. IL-12 is a critical cytokine for proliferation and activation of type 1 helper T (Th1) cells.[51] IL-23 plays an essential role to maintain Th17 cells,[52] the important involvement of which in autoimmune diseases has been shown.[53] A previous Turkish study suggested that patients with TAK displayed a higher level of IL-12p40 in their serum than a healthy population.[54] Future study should be addressed on correlation of IL-12p40 levels and disease activity. Interestingly, *IL12B* is also associated with psoriasis, inflammatory bowel diseases and leprosy.[55–58] In particular, rs6871626, the strongest susceptibility single nucleotide polymorphism (SNP) in our study, is the same SNP associated with ulcerative colitis (UC) and leprosy. However, the risk allele is common for TAK and UC but opposite for leprosy. These results suggest that genetic studies confirmed the importance of Th1 and/or Th17 in pathophysiology in TAK.[59] The suggestive association between *PSMG1* and TAK may also support overlapping of genetic factors between TAK and UC.[60] Since the neighbors of *MLX* in chromosome 17 are located in a gene-rich region,[61] it is unclear whether *MLX* is the gene responsible for TAK susceptibility. Dense

mapping combined with functional analyses may reveal the true responsible gene in this region. The involvement of *FCGR2A/3A* with TAK in a European population suggests the importance of immune-complex in pathophysiology of TAK. It is interesting because previous studies have not confirmed the importance of autoantibody or B cell functions in TAK pathophysiology.[59] Macrophages and neutrophils expressing FCGR2A and 3A, are found in the aorta lesions of patients.[62] There have also been other genetic studies, but all of them addressed HLA alleles or non-HLA markers through candidate gene approaches. *TNF-alpha*, *MYD88*, *PDCD1*, *PTPN22* and *IL12B* genes were examined,[63–67] but the *IL12B* gene was the only one demonstrating a suggestive association. We have listed a summary of genetic studies for TAK in Table 1. It should be noted that most of the studies except for the two GWAS contained less than 200 subjects. This illustrates the difficulty in collecting samples due to the relatively low prevalence of the disease. Since recent GWAS shifted to trans-ethnic or multi-ethnic meta-analysis, summing up subjects from around the world would lead to the identification of multiple susceptibility genes to this disease. It is quite interesting that TAK and leprosy, a chronic infectious disease caused by *Mycobacterium leprae*, one of the mycobacterium species, share the same SNP in relation to their susceptibility. TAK has been believed to be one presentation of tuberculosis, an infection caused by *M. tuberculosis*.[68,69] This belief is based on distribution of TAK as well as basic findings of involvement of tuberculosis with processes of vasculitis. TAK is frequently observed in East Asia or South East Asia and Turkey, where tuberculosis is widely spread. There are case reports of co-occurrence of these diseases.[70,71] Granulomatous lesions are observed in both diseases and granulomatous lesions with giant cells in TAK resemble tuberculosis follicles. There are reports of high frequency of positive tuberculin reaction in patients with TAK.[72] Furthermore, rabbit models injected with antigens of *M. tuberculosis* in the para-aortic lymph node develop symptoms resembling TAK. However, several reports revealed that there was no evidence for increase of previous infection of tuberculosis in patients with TAK compared with the general population.[73,74] Thus, although infections including mycobacterium infection may trigger TAK inflammation, there is no confirmed microbial evidence preceding TAK. Recently, Soto *et al.* revealed that IS6110 sequence, which discriminates *M. tuberculosis* from *M. bovis*, was detected in 70% of aorta specimen from patients with TAK,[75] supporting the involvement of *M. tuberculosis* with TAK processes. Exposure to *M. tuberculosis* may be sufficient to trigger TAK inflammation. Other infectious stimulations inducing TAK have also been suggested, including hepatitis B virus.[76]

Table 1 Genetic studies for Takayasu arteritis (TA) since 1990 recruiting more than 40 TA subjects

Author	Approach	Positive/ negative	Gene	No. of cases	No. of controls
Terao *et al.*[50]	GWAS	+	*IL12B, MLX, HLA-B*	379	1985
Saruhan-Direskeneli *et al.*[41]	GWAS	+	*FCGR2A/3A, IL12B, HLA-B/ MICA, HLA-DRB1/HLA-DQB1*	451	1115
Terao *et al.*[38]	Candidate	+	*HLA-B*67:01, HLA-B*52:01*	173	2000
Takamura *et al.*[48]	Candidate	+	*HLA-B*67, HLA-B*52*	96	371
Sahin *et al.*[34]	Candidate	+	*HLA-B52*	330	210
Direskeneli *et al.*[65]	Candidate	–	*PDCD1*	229	193
Lv *et al.*[67]	Candidate	–	*TNF-alpha*	110	362
Lv *et al.*[39]	Candidate	+	*DPB1*09,DPB1*1701*	72	180
Chen *et al.*[66]	Candidate	–	*MyD88*	90	270
Sahin *et al.*[63]	Candidate	–	*PTPN22*	181	177
Soto *et al.*[71]	Candidate	+	*HLA-B39,B44,B52*	40	171
Saruhan-Direskeneli *et al.*[64]	Candidate	+/–	*IL12,IL2,IL6*	94	108
Shibata *et al.*[101]	Candidate	+	*NFKBIL1*	84	217
Kimura *et al.*[102]	Candidate	+	*C1–2-A, MIB, C1–3-1*	91	248
Kimura *et al.*[103]	Candidate	+	*MICA*	81	160
Mehra *et al.*[33]	Candidate	+	*HLA-B51,B52*	80	289
Yoshida *et al.*[36]	Candidate	+	*HLA-B52, B39.2*	64	156

Studies are listed according to the reported years.

Involvement of HLA genes with TAK susceptibility indicates involvement of antigen recognition through HLA to induce inflammation in large vessels. There is a study addressing clonality of infiltrating lymphocytes in the aorta. Seko *et al.* revealed oligo clonal T lymphocytes infiltrating adventitia media in patients with TAK, suggesting that a limited antigen of the aorta is responsible for induction of activation of self-reactive lymphocytes. Furthermore, Eichhorn *et al.* showed that target molecules of autoantibodies in patients with TAK are located in the cytoplasm of endothelial cells by immunohistochemical staining.[77] Thus, there is a possibility that certain stimulation, probably infections, induces vessel inflammation through molecular mimicry recognized by HLA-B binding grooves where the 67th and 171st amino acids are especially critical.

Animal models

Although there are no established animal models for this vasculitis, several animal models develop TAK-resembling symptoms. Balb/c mice are reported to develop spontaneous aortitis.[78] Interferon (IFN)-gamma receptor deficient (IFNgammaR-/-) mice develop severe large-vessel panarteritis after herpes virus (HV) 68 infection.[79] Gamma HV68 antigen in arteritis lesions and strong tropism of gammaHV68 for smooth muscle cells were reported. This model might indicate that viral infection could lead to aortitis through the similarity of the antigens and that IFN-gamma is important for protection against aortitis. IL-1Ra deficient mice develop resemblance of autoimmune diseases in humans, including aortitis, arthritis and skin manifestations.[29] Their presentation resembles TAK, RA and psoriasis. As mentioned in the genetics section, TAK and psoriasis are suggestive of sharing genetic components to some extent. Thus, this model seemed also to support overlapping mechanisms underlying these two diseases. In this model, IL-17 and TNF-alpha are shown to play critical roles on developing autoimmune features. Aortitis and arthritis are greatly suppressed in conditions without IL-17 or TNF-alpha. As biological agents targeting TNF-alpha were reported to be effective in patients with TAK even with high disease activity, this model would give evidence of association between TNF-alpha and TAK progression. Other micro-organism infections, including *Chlamydia* pneumonia are reported to induce aortic inflammation.[80,81] Vascular involvement was not reported in IL-12B deficient mice,[82] but the antiangiogenic effect of IL-12 is widely reported.[83,84] IL-12-expressing tumor cells show low metastasis ability. In fact, IL-12/23 deficient endothelial cells showed rapid wound healing.[85] Thus, high levels of IL-12p40 in patients with TAK may prevent endothelial cells from healing from inflammation. Vascular involvement was not reported in FCGR2A or 3A deficient mice. However, a recent study reported that gene expression analysis of endothelial progenitor cells from a vascular disease rat model revealed a marked increase of FCGR2A expression.[86]

Pathophysiology

Although exact mechanisms underlying TAK are still unclear, recent reports have made much progress in the understanding of the pathophysiological aspects of this disease. Basic involvement of the aorta can be found in adventitia media and inflammatory lesions can be found in the vaso vasorum of adventitia media.[87] Thus, activation of vaso vasorum endothelial cells followed by recruitment of lymphocytes should be involved in the process of TAK. Infiltrating cells in adventitia media are composed of natural killer (NK) cells, $CD4^+$ T cells, $CD8^+$ T cells, γδT cells, macrophages and neutrophils.[62] Pathological findings based on aortic tissues from patients revealed that NK cells and γδT cells are involved with apoptosis of endothelial cells through production of perforin and killer cell lectin-like receptor subfamily K (NKG2D). Among $CD4^+$ T cells, Th1 cells secreting IFN-gamma are deeply involved with the pathophysiology of TAK. IFN-gamma promotes the formation of granulomatous lesion and giant cells.[88–90] Peripheral T cells in patients with TAK were reported to be in active state with increased CD4/CD8 ratio, suggesting dominant Th cells.[91] A recent finding also showed Th17 cells are involved with the pathophysiology of GCA, suggesting the involvement of Th17 in TAK pathogenesis.[92,93] Notch signaling was also suggested to be involved with GCA.[94] Apoptotic signaling molecules are highly expressed in endothelial cells and NK cells. Adventitia media of the aorta in patients with TAK was reported to highly express major histocompatibility complex class I and II and intracellular adhesion molecules.[20,62]

B cell involvement of TAK remains controversial. Anti-epithelial cell antibodies (AECA) and anti-aorta antibodies were reported to be found in patients with TAK.[95] In spite of several reports of functional involvement of AECA, its effect is still under controversy.[96–99] There are also reports that TAK patients often having anti-phospholipid antibodies.[100] However, the positivity of these autoantibodies and the functional meaning remain unclear.

Taken together, recent study results have elucidated the basics of TAK much more than before. Novel thera-

pies, including biological agents, are now being tried for refractory TAK. However, further efforts to collect samples and information by a standardized method are necessary to improve the prognosis of patients with TAK.

CONFLICT OF INTEREST

No competing interest exists.

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MILESTONE

History of Takayasu arteritis and Dr. Mikito Takayasu

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Takayasu arteritis (TAK) is a vasculitis mainly affecting the aorta and its large branches.[1] TAK predominantly affects young women and 90% of TAK patients are females. While many patients with TAK have been found in Asian countries, this disease is reported from all over the world. TAK is, however, seen more commonly in Indian subcontinent, Japan, China, Korea and southeast Asian countries amongst the APLAR nations as well as in Turkey, Brazil, Mexico and south Africa. Reports from western world including Iceland and USA also do exist in literature. Much about Dr. Mikito Takayasu, who was the first to report a patient with TAK, is not widely known. In this essay, Chikashi Terao from Kyoto would like to introduce the history of TAK and Dr. Takayasu.

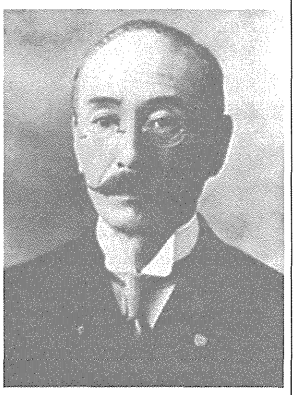

Figure 1 Portrait of Dr. Mikito Takayasu.

HISTORY OF TAKAYASU ARTERITIS

In April 1908, Dr. Mikito Takayasu (Fig. 1), a professor of ophthalmology at the current Kanazawa University, at the 12th Annual Meeting of Japanese Ophthalmology Society held in Fukuoka, reported a case of a 22-year-old woman. He described 'a case of peculiar changes in the central retinal vessels'.[2] He saw the female patient for the first time in May 1905. She had felt lowering and blurring of her vision since September 1904. She sometimes developed redness of conjuctiva. While the symptoms transiently improved by medication, they recurred in March 1905 and she visited Dr. Takayasu. She did not have a history of severe medical or gynecological diseases. While he described that she looked as if she suffered from tuberculosis (TB), he and his colleagues in the Department of Internal Medicine did not find any evidence of infection, including TB or syphilis. Her pupils were slightly dilated and the light reflex was damaged. He found eminent abnormalities in retinal vessels. Retinal vessels branched 2–3 mm away from the optic disc and the branches formed anastomosis with one another to create circularity around the disc. The branches branched further radially and the peripheral portions were narrow. The distal parts of the branches made aneurysms, anastomosis with other branches to form circularity, or terminated in a blind end. The optic disc was severely congested and hemorrhage was found around the disc. While these abnormalities were mainly found in arteries, veins anastomosed with arteries and venous blood flowed into the lesions. While he found these abnormalities in the oculus dexter, similar findings were reported in the oculus sinister. She was admitted and took medications. During the first admission, she was found to have cataracts and underwent an operation. However, her vision did not improve. She stopped visiting the hospital for several years after discharge. She revisited Dr. Takayasu's clinic in February 1908. She developed retinal detachment and her left pupil was remarkably

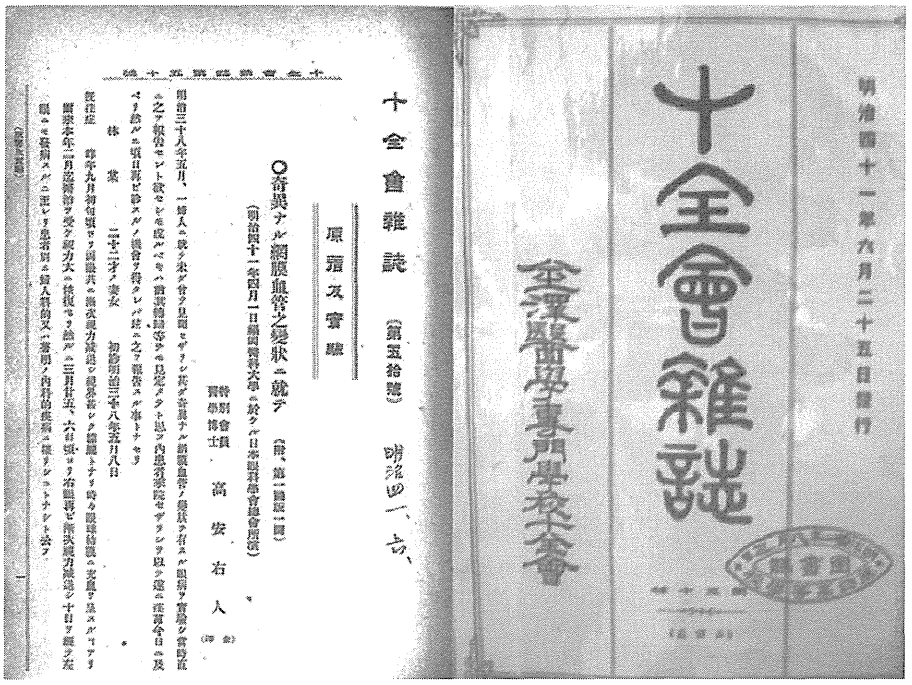

Figure 2 The manuscript of the first case with Takayasu arteritis by Dr. Mikito Takayasu.

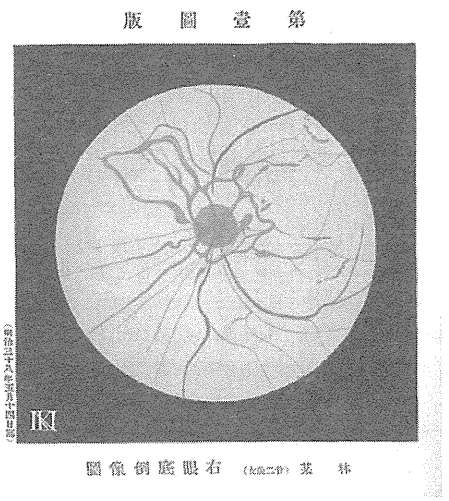

Figure 3 Hand sketch by Dr. Mikito Takayasu for the inverted image of fundus in the oculus dexter from the first case.

dilated. Dr. Takayasu had no choice but to tell her that there was no effective treatment. He described that the anastomosis and aneurysmal changes should be the primary findings and that the others should be the secondary findings. After his presentation at the Congress, Dr. Yoshiakira Ohnishi, a professor at Kyushu University, mentioned another female case resembling the case presented by Dr. Takayasu. He described that he could not sense her pulse at the bilateral radial arteries. This case report by Dr. Takayasu was published in June in the same year in the *Journal of the Juzen Medical Society* in Kanazawa University (Fig. 2). In this manuscript, he presented an image of the arteries that he beautifully hand-sketched himself (Fig. 3).

While it is often regarded that it was Dr. Takayasu who first reported a patient with TAK, there are other prior potential case reports of patients with TAK. Giovanni Battista Morgagni, an Italian anatomist, described a 40-year-old woman suffering from pulseless disease.[3] In 1830, Rokushu Yamamoto described a 45-year-old man suffering from fever.[4] After 1 year, the patient became pulseless in the right radial artery and had a very weak pulse in the left radial artery. The patient developed pulseless disease in both carotid arteries and died 11 years after the first visit. He described two other cases reported by another doctor. In 1856, Savory[5] reported a 22-year-old woman presenting with pulseless disease in both upper extremities and left neck. The patient lost her vision. However, whether these cases truly suffered from TAK is uncertain. Numano[4] suspected the case reported by Savory should be explained

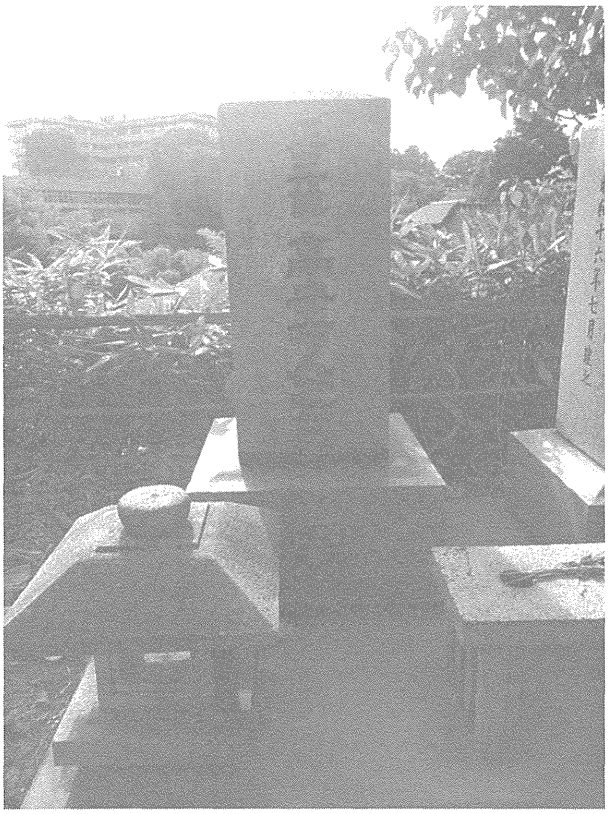

Figure 4 Dr. Mikito Takayasu's grave in the Hoenji Temple near Kanazawa University.

Figure 5 A statue of Dr. Mikito Takayasu in Kanazawa University.

by other diseases since the patient had a corneal ulcer leading to invasion of scalp and brain.

Dr. Minoru Nakajima in 1921 compared his cases with previous reports and proposed that they should be regarded as one disease. He characterized this disease by the following four criteria: (i) affecting bilateral eyes in young women; (ii) arteriovenous anastomosis around the optic disc and microaneurysm formation in retinal vessels; (iii) lowering of vision complicated with cataract; and (iv) unpalpable radial artery. He proposed to call this disease 'Takayasu disease'. After his proposal, Japanese ophtalmologists paid attention to this disease and many cases were reported. In 1946, Frövig[6] proposed that the diseases presenting pulselessness should be called 'aortic arch syndrome'. In 1948, Drs Kentaro Shimizu and Keiji Sano, brain surgeons at the University of Tokyo, proposed to give an alias name of 'pulseless disease'. In 1951, this disease was reported by these doctors outside of Japan for the first time, by reviewing a total of 25 cases.[7] Since 1951, there have been case reports from a number of foreign countries. In 1952, Caccamise and Whitman[8] reported the primary occidental case report. In 1962, Judge *et al.*[9] described 'Takayasu's arteritis'. In Japan, many doctors proposed acronyms of TAK. Drs Maekawa and Kakei called it 'occlusive coagulant aortic syndrome'.[10] Dr. Nasu[11] called it 'obstructive productive arteritis'. In 1965, Riehl *et al.*[12] analyzed this disease from pathological and immunological aspects and proposed the concept that this disease is an autoimmune disease. Since 1990 when the American College of Rheumatology published a classification criteria of TAK[13] and described 'Takayasu arteritis', the name of TAK prevailed worldwide. Although both 'Takayasu arteritis' and 'Takayasu's arteritis' are used, in the Online Mendelian Inheritance in Man (OMIM) it is registered as 'Takayasu arteritis' and this expression is more commonly used.

The case report by Dr. Takayasu is quite suggestive. His report tells us that we should carefully examine and observe patients to find characteristics of the patients. Even if we saw just one case, there is a possibility that many patients exist with the same symptoms. It is

impressive that Dr. Takayasu described the first patient as being similar to patients with TB. In fact, there are studies pointing out the overlapping of TB and TAK.[14,15] Geographical prevalence of TAK is similar to that of TB. Although there are many conflicting studies in terms of epidemiology and immunology,[16] there is a possibility that exposure to TB triggers the immune reaction to TAK. In fact, my group showed that the susceptibility variant is located in the *IL12B* region[17] and this variant has previously been reported in relation to mycobacterium infection.

HISTORY OF MIKITO TAKAYASU

Dr. Mikito Takayasu was born on 19 July, 1860 in Saga prefecture in Japan as the fourth son of a priest. He graduated from Tokyo foreign language school and entered Tokyo University. He graduated from Tokyo University in 1887 and soon moved to Kanazawa prefecture as a lecturer. He went to Germany for 2 years, where he performed research in Berlin City Hospital, Berlin Charité University Hospital and Leipzig University. He studied under Dr. von Graphe. He showed by means of Sudan dyes that arcus senilis occurs due to fat deposition.[18] Until his findings, little was known about the nature of arcus senilis in spite of the high frequency of this phenomenon. He returned to Kanazawa and obtained his PhD degree in 1903 for this research. He then went on to become a professor and principal of the Medical School. In 1923, the Medical School was reorganized as the Medical University and he was appointed Dean. He retired from the University in 1924. After retirement, he opened his own clinic near the University Hospital. To prevent the lowering in numbers of patients in surrounding ophthalmologist clinics, he set a very expensive doctor's fee. Despite the expensive fees, his clinic was very popular because patients highly appreciated his sound character and respected his medical skills, experience and knowledge. It is said that many people believed that water from the small river running by his house was effective for eye diseases. In 1933, he suffered from stroke and moved to Beppu in Kyushu for recovery. He died of rectal cancer in Beppu when he was 78 years old on 20 November, 1938. His bones were transferred to Kanazawa soon after his death and his funeral was conducted at Kanazawa University. His grave has been kept in the Hoenji Temple near Kanazawa University (Fig. 4). His statue was built in Kanazawa University in 2002 (Fig. 5). He had three sons and five daughters. Dr. Akira Takayasu, his second son, was a professor of ophthalmology at Kagoshima University. Dr. Tatsuo Hirose, his great-grandson, is a clinical professor of ophthalmology at Harvard Medical School. Dr. Hirose is a grandson of Ms. Miyako Takayasu Hirose, one of Dr. Takayasu's daughters. According to Dr. Hirose, he had seen a couple of young women with TAK during his 4-year stay in the Ophthalmology Clinic in Kanazawa University. However, he has not seen a single patient with TAK in Boston during his career over 40 years in a tertiary referral retina clinic in Boston. This can be explained by different prevalences of TAK among different populations.

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ORIGINAL ARTICLE

Effects of Smoking and Shared Epitope on the Production of Anti–Citrullinated Peptide Antibody in a Japanese Adult Population

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Objective. **Anti–citrullinated peptide antibody (ACPA) and rheumatoid factor (RF) are markers to rheumatoid arthritis (RA). Smoking and shared epitope (SE) in HLA–DRB1 are associated with the production of these autoantibodies in RA. Detailed distribution and characterization of ACPA and RF in the general population have remained unclear. We aimed to evaluate positivity of ACPA and RF in a general Japanese population and to detect correlates, including genetic components.**

Methods. **ACPA and RF were quantified in 9,804 Japanese volunteers ages 30–75 years. Logistic regression analyses were performed to evaluate the effects of candidates of correlates on the autoantibody positivity. A genome-wide association study (GWAS) was performed using 394,239 single nucleotide polymorphisms for 3,170 participants, and HLA–DRB1 alleles were imputed based on the GWAS data.**

Results. **A total of 1.7% and 6.4% of subjects were positive for ACPA and RF, respectively, and the 2 markers showed a significant correlation ($P = 2.0 \times 10^{-23}$). Old age was associated with ACPA positivity ($P = 0.00062$). Sex, smoking, SE, and other candidates of correlates did not have significant effects. Interaction between smoking and SE positivity was not apparent, but smoking showed a significant association with high levels of ACPA ($P = 0.0019$).**

Conclusion. **ACPA and RF could be detected in 1.7% and 6.4% of the Japanese adult population without RA, respectively. ACPA and RF were suggested to share mechanisms even in healthy populations. Old age was associated with increasing ACPA positivity. While positivity of ACPA and RF was not associated with SE and smoking, an association between high ACPA and smoking was observed.**

INTRODUCTION

Rheumatoid factor (RF), an IgM autoantibody against the Fc fraction of IgG, is a serum marker of rheumatoid arthritis (RA) (1,2). In spite of its specificity to RA, RF appears in other diseases, especially connective tissue diseases, hepatic disorders, and even in healthy populations (3–9). Recently, anti–citrullinated protein antibody (ACPA) was found to show high specificity to RA and was able to distinguish RA from other connective tissue diseases with higher accuracy compared with RF (1,10). Although some studies reported functional pathogenicity of ACPA (11), pathogenicity and production mechanisms of ACPA and RF are largely unknown. Vigorous studies that address associations with the positivity and levels of ACPA and RF in patients with RA identified a wide range of factors. Some are disease-specific factors, such as disease

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Significance & Innovations

- Positivity of anti–citrullinated peptide antibody (ACPA) in the general population is associated with aging and high C-reactive protein level.
- Smoking and shared epitope do not have comparable effect in the general population on the production of ACPA and rheumatoid factor (RF) as with patients with rheumatoid arthritis.
- Smoking may be associated with a high level of ACPA, even in healthy subjects.
- Correlates should be taken into account for RF and ACPA positivity in the general population. Novel findings of RF and ACPA production in general populations would provide clues to uncover the pathophysiology of the production of these autoantibodies.

activity and extraarticular symptoms (12–14) and others are disease–non-specific factors such as age, smoking, and common variants of HLA alleles (8,15–17). Smoking was shown to have an effect on the susceptibility to seropositive RA, especially in men (18). HLA–DRB1 is the strongest susceptibility locus to RA and is associated with ACPA or RF positivity in patients with RA (19). In particular, shared epitope (SE), an allelic group with a common amino acid pattern from the 70th to the 74th amino acid of the HLA–DRB1 protein (20), is strongly associated with RA susceptibility and production of ACPA and RF in patients with RA (15,17).

However, the distribution of these antibodies and whether the correlates are associated with positivity of ACPA or RF in the general population is largely unknown. There are no reports where ACPA levels were quantified and correlates of ACPA were analyzed in a large-scale study of healthy individuals. Although there are reports suggesting that the positivity of RF in healthy individuals is influenced by age and smoking in a European population (8,21–25), the positivity of RF and its correlates in healthy individuals is not known in Asian populations. If the likelihood of having RA based on positivity of ACPA or RF is different between subgroups with and without correlates, determining the distribution and correlates of ACPA and RF in a healthy population would lead to efficient screening to identify subjects at risk of RA. Moreover, determining the distribution and correlates would give clues for novel insights of mechanisms of production for ACPA and RF.

Here, we quantified circulating levels of ACPA and RF in 9,804 healthy Japanese subjects, identified prevalence, and estimated correlates, including genetic factors, of these 2 autoantibodies.

PATIENTS AND METHODS

Study population. This study was conducted as a part of the Nagahama Prospective Genome Cohort for Comprehensive Human Bioscience (The Nagahama Study) (26), a community-based prospective multiomics cohort study conducted by Kyoto University. A total of 9,804 volunteers in Nagahama City, Shiga Prefecture, Japan were recruited in this study from 2008 to 2010. All participants were asked to complete a detailed questionnaire about their present symptoms, present illness, past history of illness, family history, and smoking status. Written informed consent was obtained from all of the participants. This study was approved by Kyoto University Graduate School and Faculty of Medicine Ethics Committee.

Exclusion of samples. We excluded volunteers from the association studies if they had or have had autoimmune diseases. Individuals who were judged from their answers to the questionnaire to possibly have autoimmune diseases were also excluded from the analyses. As a result, a total of 9,575 subjects were recruited for the analysis.

RA patients. A total of 2,067 patients with RA in Tokyo Women's Medical University, whose age at onset, sex, and data of ACPA and RF were available, were registered in this study. A total of 1,237 patients with RA in Kyoto University were used for correlation analysis of genetic components.

Quantifying of circulating autoantibody. Serum samples were obtained from all the participants. ACPA was quantified as second-generation anti–cyclic citrullinated peptide (anti-CCP) antibody by MesaCup CCP enzyme-linked immunosorbent assay kit (Medical and Biological Laboratories) (27,28). IgM-RF was quantified by latex turbidimetric immunoassay, Iatro-RF II (Mitsubishi Kagaku Medience) (29). Both autoantibodies were quantified by SRL for healthy individuals and in Tokyo Women's Medical University for patients with RA. The cutoff levels of the autoantibodies were according to manufacturer's instructions (ACPA <4.5 units/ml, RF ≤ 20 IU/ml).

Candidates of correlates for ACPA and RF. Age, sex, smoking status, Brinkman index (BI; number of cigarettes a day $\times$ smoking years) as a quantitative measure of smoking, alcohol consumption, body mass index (BMI), and serum level of C-reactive protein (CRP) were selected as candidates of correlates for ACPA and RF. They were selected based on the previous reports of significant association between RA and smoking and a study from the US analyzing correlates of anti–nuclear antibody in the general population (30). We classified all the included participants into 5 groups according to their age at 10-year intervals. Logistic linear regression analysis or chi-square test was performed to analyze the influence of candidates of correlates on the positivity of autoantibodies. The effects of smoking in conditions with alcohol consumption were also analyzed.

Genome-wide association study (GWAS). GWAS was performed for 3,710 samples of participants who joined the Nagahama Study during 2008 to 2009. A series of BeadChip DNA array was used for the genotyping and