日本における薬剤性肺障害の臨床像

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薬剤性肺障害のなかで最も頻度が高い病態は間質性肺炎である。われわれは薬剤性間質性肺炎の臨床像を解析するために，全国の病院より症例を収集しており，現在までに152例の解析を行った。自覚症状は呼吸困難，発熱，咳嗽の順で多かったが，無症状の例も15例認めた。原因薬剤としては抗悪性腫瘍薬が81例で最多であった。薬剤の投与期間は2～8,280日で，中央値は60日であった。血清KL-6の平均値は968U/mLと増加していたが，正常範囲の症例も33例に認めた。治療としては146例で被疑薬が中止され，122例でパルス療法を含む副腎皮質ステロイドが使用された。予後は139例が治癒もしくは軽快であったが，3例の死亡例も認めた。

Key word 薬剤性肺障害，薬剤性間質性肺炎，抗悪性腫瘍薬，分子標的薬，副腎皮質ステロイド

薬剤性肺障害のわが国における現状

近年，分子標的治療薬や生物学的製剤の相次ぐ開発・上市などがあり，薬剤性肺障害を来す薬剤は増加傾向である[1]。薬剤性肺障害の診断として，次のような基準が提唱されている[2]。①原因となる薬剤の摂取歴がある，②薬剤に起因する臨床病型の報告がある，③他の原因疾患が否定される，④薬剤の中止により病態が改善する，⑤再投与により増悪する。しかし，③の他疾患の否定は必ずしも容易ではなく，④の薬剤中止による改善は，発症時には判断できない。さらに⑤の再投与による増悪は，倫理的側面から通常は行われない。薬剤性肺障害に特異的な身体所見や検査所見はない。すなわち，薬剤性肺障害は除外診断であり，病歴，自覚症状，血液検査，画像所見および気管支肺胞洗浄所見などを総合した臨床診断に頼らざるをえない。

薬剤性肺障害は原因薬剤の中止のみで改善する例が多いが，死亡例も少なからず存在する。特にわが国では薬剤性肺障害の発症頻度，および死亡率が諸外国と比較して高いとされており[3]，その臨床像を明らかにする必要がある。しかし薬剤性肺障害は比較的発症頻度の低い疾患であり，全国レベルでの症例集積が望ましいと考えられる。

なお薬剤性肺障害には間質性肺炎や気道病変，血管病変などさまざまな病型があるが，本稿では最も高頻度に認める薬剤性間質性肺炎について述べる。

薬剤性間質性肺炎の症例収集

われわれは2009～2011年度に厚生労働科学研究費補助事業の一環として，千葉大学，広島大学，社会保険中央総合病院（現 東京山手メディカルセンター）と共同で薬剤性間質性肺炎の症例を収集してきた。さらに2012年度からは同様の研究事業に日本医科大学および国立医薬品食品衛生研究所も参加し，全国の病院より症例を集積するシステムとなった（図1）。症例を収集するために，

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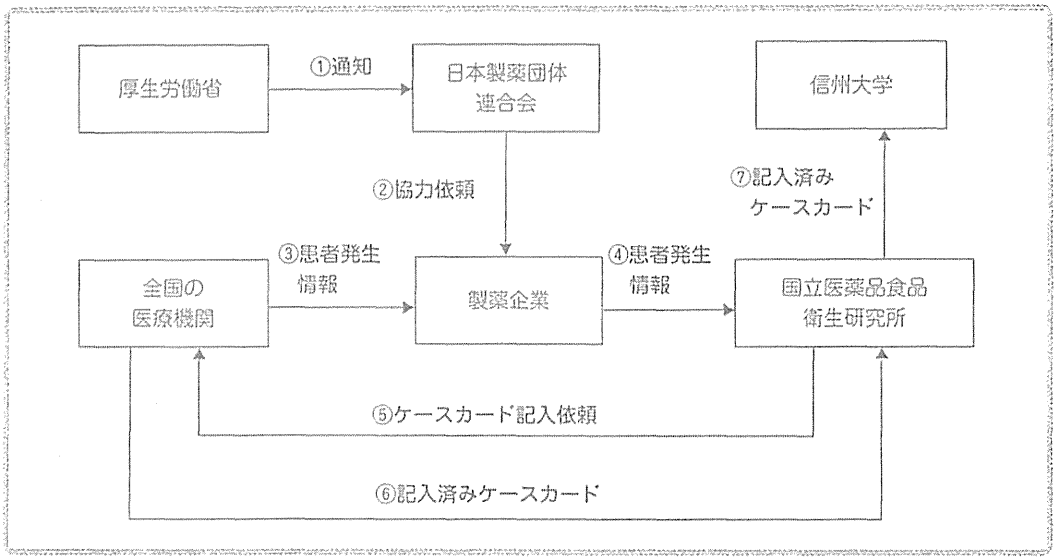

図1　薬剤性間質性肺炎の症例収集の流れ

厚生労働省より日本製薬団体連合会を通じて，各製薬企業への本研究への協力依頼を行った（図1①，②）。全国の医療機関より薬剤性肺障害患者の発生が医薬品医療機器総合機構を通じて各製薬企業へ届出されると，その届出が国立医薬品食品衛生研究所にも報告される（図1③，④）。国立医薬品食品衛生研究所は，患者が発生した医療機関へ患者の臨床情報を記載するケースカードを郵送し，記入の依頼を行う（図1⑤）。医療機関の医師は患者より同意を取得後にケースカードを記載し，国立医薬品食品衛生研究所へケースカードを返送し（図1⑥），さらにケースカードは信州大学へ郵送される（図1⑦）。そして最終的に信州大学で薬剤性間質性肺炎の臨床像の解析が行われる流れとなっている。

薬剤性間質性肺炎の臨床像

全国の医療機関で発生した薬剤性間質性肺炎152例の臨床像は以下のとおりであった。男性110例，女性42例で，平均年齢は67.8歳であった。自覚症状としては呼吸困難62例（40.8％），発熱51例（33.6％），咳嗽49例（32.2％）が多かったが，無症状の例も15例（9.9％）認めた（表1）。原病，合併症もしくは既往歴として呼吸器疾患を有する症例は53例（34.9％），喫煙歴を有する症例は90例（59.2％）であった。

表1　自覚症状（重複あり）

	症例数（割合）
呼吸困難	62（40.8％）
発　熱	51（33.6％）
咳　嗽	49（32.2％）
食欲低下	8（5.3％）
喀　痰	7（4.6％）
な　し	15（9.9％）

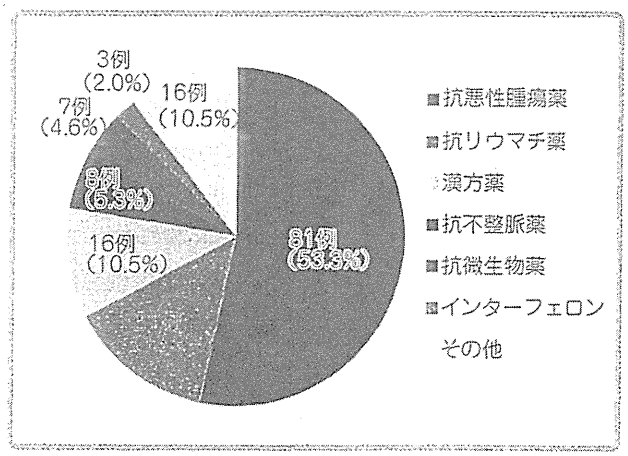

図2　原因薬剤別の症例数

原因薬剤は抗悪性腫瘍薬81例（53.3％），抗リウマチ薬21例（13.9％），漢方薬16例（10.5％），抗不整脈薬8例（5.3％），抗微生物薬7例（4.6％），インターフェロ

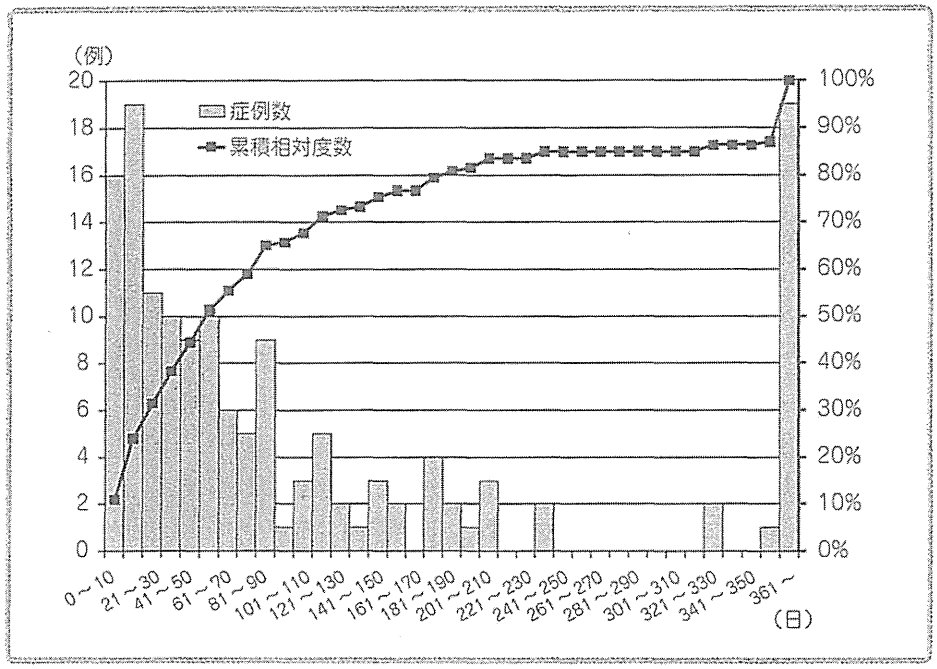

図3　薬剤投与から発症までの日数

ン3例（2.0%），その他16例（10.5%）であり，抗悪性腫瘍薬が過半数を占めた（図2）。分子標的薬に分類される薬剤が原因であった症例は35例（23.0%）であった。薬剤投与開始から発症までの期間は2～8,280日で，中央値は60日であった。投与後60日以内に過半数の症例が，140日以内に75%の症例が発症していた。投与開始後360日以降の発症は19例（13.0%）であった（図3）。

身体所見では，SpO_2が90%以上の症例は62例（40.8%），90%未満の症例は28例（11.1%）であったが，未測定の症例も62例（40.8%）あった。胸部聴診所見では捻髪音を58例（38.2%）に，水泡音を6例（3.9%）に，笛声音を3例（2.0%）に，いびき音を2例（1.3%）に認めた。胸部聴診上，ラ音が聴取されない症例は25例（16.4%）であった。しかし，胸部聴診所見が不明の症例も56例（36.8%）あった。

血液検査では白血球数の平均は7,954.2/μLとほぼ正常であったが，CRPは6.45mg/dLとやや増加していた。また，種々の間質性肺炎の際に上昇することが多いLDHの平均値は308.6 IU/Lと本検討でも上昇していた。比較的間質性肺炎に特異的とされる血清KL-6，SP-D，SP-Aはそれぞれ135例，76例，26例で測定され，その平均値は968U/mL，183.7ng/mL，77.5ng/mLと増加していた（表2）。KL-6，SP-D，SP-Aが正常範囲の症例もそれぞれ33例，30例，4例認めたが，3つの検査値すべて正常の症例は1例のみであった。

表2　血清学的検査

検査項目（単位）測定症例数	平均値±標準偏差
KL-6 (U/mL) 135例	968±1,118
SP-D (ng/mL) 76例	183.7±206.8
SP-A (ng/mL) 26例	77.5±32.8

胸部高分解能CTによる画像検査では，115例（75.7%）が両側性のすりガラス影，浸潤影であったが，片側性の陰影の症例も12例（7.9%）認めた。

呼吸機能検査で肺気量分画は18例で測定されており，予測値に対する肺活量の割合の平均値は89.2%，1秒量の平均値は79.5%と正常範囲であった。拡散能まで測定してあった症例は14例で，予測値に対する拡散能の割合の平均値は63.6%と低下していた。

表3　治療（重複あり）

	症例数（割合）
被疑薬の中止	146（96.1％）
副腎皮質ステロイド薬	122（80.3％）
シベレスタット	7（4.6％）
シクロスポリン	2（1.3％）
エンドトキシン吸着療法	1（0.7％）

65例に気管支肺胞洗浄（bronchoalveolar lavage：BAL）検査が施行され，平均総細胞数は3.8×10⁶/mLと増加していた。細胞分画ではリンパ球増多（15％以上）を51例，好酸球増多（1％以上）を47例，好中球増多（3％以上）を34例で認め，多彩な所見であった。薬剤によるリンパ球刺激試験（drug-induced lymphocyte stimulation test：DLST）は68例に施行され，29例で陽性であった。

治療としては146例（96.1％）で被疑薬が中止され，122例（80.3％）でパルス療法を含む副腎皮質ステロイド薬が使用された。さらに重症例ではシベレスタットが7例（4.6％）で，シクロスポリンが2例（1.3％），エンドトキシン吸着療法が1例（0.7％）で併用された（表3）。呼吸管理として非侵襲的陽圧換気を要した症例は6例（3.9％）で，気管内挿管し，人工呼吸管理を行った症例は3例（2.0％）であった。

予後は139例（91.4％）で治癒もしくは軽快と比較的良好であったが，3例（2.0％）が死亡した（図4）。

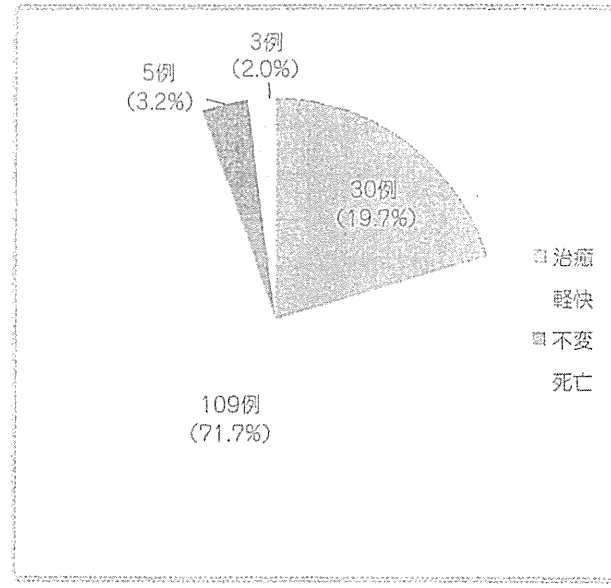

図4　予後

臨床像のまとめ

今回の検討から得られた薬剤性間質性肺炎の臨床像を以下に列挙する。

①原因となる薬剤投与後，（過半数の症例が60日以内に）呼吸困難，咳嗽，発熱で発症する。

②原因薬物は多彩であったが，抗悪性腫瘍薬が高頻度であった。また分子標的薬も比較的高頻度であった。

③ほぼすべての症例で血清KL-6，SP-D，SP-Aのうちいずれかが増加していた。

④高分解能CTでは両側性にすりガラス影や浸潤影を認める例が多かった。

⑤BALの細胞分画に一定の傾向はなかった。

⑥DLSTは68例中29例で陽性であった。

⑦被疑薬の中止や副腎皮質ステロイド投与によりほとんどの症例が改善したが，3例（2.0％）の死亡例も認めた。

すなわち薬剤性間質性肺炎の臨床像は多彩であり，確定診断は必ずしも容易ではない。基礎疾患である肺病変の悪化や，感染症などと十分に鑑別する必要がある。

今回の検討からは，抗悪性腫瘍薬など高頻度に薬剤性間質性肺炎を来す薬剤を投与されてから半年以内の経過で，胸部CTで両側性の陰影を呈し，血清学的にKL-6，SP-D，SP-Aのいずれかが増加している患者では，本症を積極的に鑑別する必要があることが示唆された。

今回検討した症例ではSpO_2 90％以上の症例が40％程度と比較的多く，さらに未測定の症例も同程度あった。未測定の症例は呼吸困難や咳嗽などの呼吸器症状の訴えがなく，比較的軽症であることが予測され，SpO_2が90％以上の症例とあわせて，本検討では比較的軽症例が多かったと考えられる。

胸部聴診所見では捻髪音を認める症例が多かった。捻髪音は間質性肺炎などのときに比較的高頻度に聴取されるラ音である。胸部X線で所見を呈さない早期の間質性

肺炎でも聴取されることがあり，間質性肺炎の早期発見に非常に有用な所見である。今回の検討では胸部聴診所見が不明の症例も少なからず存在したが，胸部の聴診は薬剤性間質性肺炎診療においても有用かつ，聴診器1本でできる診察であり，日常診療で欠かさず行いたい診察である。

DLSTは定量化により陽性基準が設定されており，一部では信頼性の高い検査として汎用されている。しかし，偽陰性率が高いことはよく知られており，この偽陰性・偽陽性の問題，検査に使用する薬剤の濃度基準の問題，不溶性薬剤の問題などいくつかの問題点が指摘されている。今回DLSTを施行した68例のうち陽性は29例のみであり，陽性率は低かった。現状では薬剤性間質性肺炎を客観的に確定できる検査は存在せず，本症の診断をより困難にしている。

治療として原因薬剤の中止はほとんどの症例で行われ，薬剤中止のみで軽快する症例も多数認めた。近年上市された分子標的薬エベロリムスは，投与された患者のうち約半数と非常な高頻度で薬剤性間質性肺炎を来す薬剤である。その一方，軽症例では投与継続，もしくは休薬後の再投与が可能とされており，原則的に再投与は禁忌とされてきた従来の薬剤性間質性肺炎の治療とは大きく異なる[3]。

今回の検討では抗悪性腫瘍薬が原因薬剤の多数を占めており，その薬剤の投与の可否は患者の予後に大きな影響を与える。したがって，除外診断ではなく薬剤性間質性肺炎を特異的に診断する方法の開発が望まれる。

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Association Between Antinuclear Antibodies and the HLA Class II Locus and Heterogeneous Characteristics of Staining Patterns

The Nagahama Study

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on behalf of the Nagahama Study Group

Objective. **While antinuclear antibodies (ANAs) are observed in healthy populations as well as in patients with autoimmune diseases such as systemic lupus erythematosus (SLE), the detailed genetic background of ANAs has remained unclear. We undertook this study to identify the genetic determinants of ANAs in the general population in order to elucidate the underlying mechanisms of ANA production and to distinguish disease susceptibility genes from ANA production genes.**

Methods. **A total of 9,575 Japanese volunteers were registered, and their ANA levels were quantified using indirect immunofluorescence to analyze correlates of ANA positivity. Genetic studies were performed using 7,148 of the 9,575 subjects. We performed a genome-wide association study using 3,185 subjects genotyped for 303,506 single-nucleotide polymorphisms (SNPs), followed by a replication study of 3,963 subjects. HLA–DRB1 and HLA–DQB1 alleles were imputed, and associations between ANA positivity and the SNPs or the HLA alleles associated with SLE were analyzed.**

Results. **Female sex and old age were associated with ANA positivity, except for the nucleolar pattern. The T allele of rs2395185 in the HLA locus, which was in moderate linkage disequilibrium with HLA–DRB1*0405, was significantly associated with ANA positivity ($P = 1.3 \times 10^{-11}$). The T allele of rs2395185 displayed increasing effects on the frequency of speckled and homogeneous patterns ($P = 7.5 \times 10^{-12}$ and $P = 2.2 \times 10^{-11}$, respectively) but decreasing effects on the frequency of the nucleolar pattern ($P = 0.0045$). The 7 SNPs and 4 HLA–DRB1 alleles associated with SLE did not display strong associations with ANA positivity.**

Conclusion. **SNP rs2395185 linked with HLA–DRB1*0405 is a genetic determinant of ANA production in the Japanese population. Overlapping of loci for susceptibility to SLE and to ANA positivity was limited. The nucleolar pattern showed different associations from other staining patterns, both with correlates of ANA positivity and with the HLA locus.**

Antinuclear antibodies (ANAs) are autoantibodies that recognize various nuclear and cytoplasmic proteins, and they are frequently observed in patients with a broad range of diseases including systemic lupus erythematosus (SLE), hepatic disease, malignant disease, lung disease, and a variety of infections (1–6). The distribution patterns of fluorescent types of ANAs (such as speckled, homogeneous, nucleolar, or discrete speck-

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led patterns) also provide useful information for differential diagnosis (7–9). Previous studies have suggested that it is not unusual to find healthy individuals who are positive for ANAs (10). Since ANAs are included in the classification criteria for SLE as well as those for autoimmune hepatitis (11,12), analyzing the kinds of variables that affect the levels of ANAs would be helpful for avoiding excessive or deficient classification of these diseases as well as for gaining insight into their etiologies.

Although previous studies showed that ANA positivity was associated with female sex, old age, and being overweight (13,14), genetic components affecting ANA positivity in healthy individuals have never been addressed. Genome-wide association studies (GWAS) have detected many genes that confer susceptibility to connective tissue diseases, including SLE (15–18), and have elucidated the genetic background of biomarkers in general populations (19). Because almost all patients with SLE are positive for ANAs, it is important to confirm that SLE-related genes in the previous GWAS were not merely derived from their associations with ANA positivity.

At present, the number of large-scale studies addressing ANA levels in healthy subjects is quite limited. Detailed analyses of the correlates and genetic components of ANAs in healthy individuals would provide clues to the mechanisms responsible for the production of autoantibodies and the development of autoantibody-mediated autoimmune diseases (20,21). In the present study, we quantified circulating levels of ANAs in 9,575 Japanese volunteers for detailed analyses of the distributions and effects of correlates on ANA production. We also performed a GWAS in 7,148 of the 9,575 subjects to detect susceptibility loci that affect ANA production.

SUBJECTS AND METHODS

This study was approved by the Ethics Committee of Kyoto University Graduate School and Faculty of Medicine.

Study population. This study was performed as a part of the Nagahama Prospective Genome Cohort for Comprehensive Human Bioscience (the Nagahama Study), a community-based prospective multiomics cohort study conducted by the Center for Genomic Medicine at Kyoto University (22). A total of 9,809 volunteers ages 30–75 years in Nagahama City, Shiga Prefecture, Japan were recruited for this study. Written informed consent was obtained from each participant, and all were asked to complete a detailed questionnaire including present and past illnesses and lifestyle.

Exclusion criteria. We excluded volunteers from the association studies if they lacked necessary information or had ever been told that they have or had an autoimmune disease. We also excluded individuals whose answers to the questionnaire suggested that they might have an autoimmune disease. As a result, a total of 9,575 subjects remained for this study. A detailed flow chart of sample exclusion is shown in Supplementary Figure 1 (available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38867/abstract).

Quantification of ANAs and C-reactive protein (CRP). ANAs and CRP in serum samples from volunteers were quantified (23) at SRL, one of the largest clinical laboratory testing companies in Japan. ANAs were quantified by serum dilution using indirect immunofluorescence with HEp-2 cells (TFB). Titers of ANAs with detailed staining patterns (speckled, homogeneous, nucleolar, cytoplasmic, and discrete speckled patterns) were also reported for these subjects. A cutoff level of 1:40 for positivity was applied according to the manufacturer's instructions.

Selection of potential correlates. Age, sex, body weight, smoking, alcohol use, and serum CRP level were selected as potential correlates based on a previous US study (14). CRP was quantified by highly sensitive methods using nephelometry, with a detection limit of 0.051 mg/liter, as previously reported (23).

Statistical analysis of nongenetic studies. The subjects were divided into 2 subgroups based on sex, 9 subgroups based on age (5-year intervals), and 18 subgroups based on sex and age. Associations between ANAs and age and/or sex were assessed by standardized logistic regression analysis. Odds ratios were also calculated with 95% confidence intervals. The associations between ANAs and potential correlates were analyzed by logistic regression analysis, with sex and age as covariates. Statistical analyses were performed using R statistical software (http://www.r-project.org) or SPSS version 18. We set significance levels in a conservative manner using Bonferroni correction for multiple testing.

GWAS. DNA samples from 3,710 of the 9,809 participants in the Nagahama Study were genome-scanned using Illumina HumanHap610, HumanHapOmni2.5-4, or Human HapOmni2.5-8 arrays. A total of 392,801 single-nucleotide polymorphisms (SNPs) that were common between the arrays were selected for the GWAS. We selected 3,185 subjects with call rates of >0.95 who did not show a high degree of kinship (PI_HAT <0.35) and who did not have connective tissue diseases. SNPs that showed P values less than 5×10^{-7} and in Hardy-Weinberg equilibrium ($P > 1 \times 10^{-7}$) with a success rate of >0.95 and a minor allele frequency of >0.05 were selected for a replication study using a TaqMan Assay (Applied Biosystems) with 3,963 of the participants. Population stratification was assessed with genomic control (24). Logistic regression analysis was performed to analyze the genetic influence on the production of ANAs for each SNP, corrected by age and sex. Logistic regression analysis was also used for the conditioning analysis. The associations of the 2 studies were combined using the inverse-variance method. The Jonckheere-Terpstra test was used to assess increasing effects of SNPs on ANA levels in subjects positive for ANAs.

HLA imputation. The HLA–DRB1 locus (the established HLA locus associated with SLE in previous reports) and the HLA–DQB1 locus were imputed using the GWAS data with HLA*IMP:02 (25). The imputation accuracy was evaluated by kappa coefficient with the use of imputation and genotyping data for 589 patients with rheumatoid arthritis and 932 healthy subjects for HLA–DRB1, as previously described

(23), and for 114 patients with thyroid diseases for HLA–DQB1 (Terao: unpublished observations). We analyzed whether each allele of HLA–DRB1 and HLA–DQB1 with imputation accuracy >70% was associated with ANA positivity by logistic regression analysis with additive or dominant models.

Evaluation of linkage disequilibrium (LD). LD between SNPs and HLA–DRB1 alleles was obtained from previous studies (17,26,27). For LD calculation between HLA–DRB1 and HLA–DQB1 alleles, we used genotyping data of 1,000 unrelated healthy Japanese subjects (Terao: unpublished observations).

Evaluation of effects of SLE-related SNPs. A total of 7 SNPs that displayed associations with SLE beyond levels significant in GWAS in a Japanese population (15) and the 5 SNPs in the HLA locus that displayed independent associations with SLE in Europeans (28) were selected to assess their effects on ANA positivity. The associations between these SNPs and ANA positivity were analyzed based on imputation by MaCH (29), using 192 samples in the Nagahama Study genotyped by HumanHapOmni2.5-8, HumanHapOmni2.5s, and HumanExome arrays or using East Asian panels in the 1000 Genomes Project as a reference when they were not directly genotyped.

Statistical analysis of genetic studies. Statistical calculations were performed using Plink software version 1.07 (30) and R statistical software. For all genetic analyses including the GWAS, we set significance levels using the Bonferroni correction for multiple testing.

RESULTS

A total of 9,575 subjects were analyzed for their ANA levels in the current study (Table 1). ANA titers in 45.2%, 12.5%, and 2.8% of the volunteers were ≥1:40, ≥1:80, and ≥1:160, respectively (see Supplementary Table 1, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38867/abstract). When we analyzed potential correlates of ANA positivity, female sex and old age had higher correlations with ANA positivity, as shown in previous studies (13,14) (corrected P [P_{corr}] $< 1.0 \times 10^{-10}$) (see Supplementary Figure 2 and Supplementary Table 2, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38867/abstract).

When we focused on each staining pattern, 43.7%, 25.3%, 4.7%, 0.9%, and 2.0% of subjects had ANAs with speckled, homogeneous, nucleolar, discrete speckled, and cytoplasmic patterns, respectively, at titers of ≥1:40 (Table 1). The multiple logistic regression analyses revealed that the nucleolar pattern was not associated with age or sex (see Supplementary Table 2 and Supplementary Figure 3, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38867/abstract). Considering the higher frequency of the nucleolar pattern compared with that of the discrete speckled pattern, these results indicated that age and sex do not influence the positivity for each staining pattern in the same manner. Positivity for the speckled pattern was strongly correlated with positivity for all ANAs (see Supplementary Figure 4, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38867/abstract). Associations between other potential correlates and ANAs are shown in Supplementary Table 3 (available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38867/abstract). High CRP levels showed an association with ANA positivity (P_{corr} = 0.0029). We did not find a significant association between obesity and ANA positivity.

Table 1. Characteristics of the subjects in the current study*

	All subjects (n = 9,575)	GWAS (n = 3,185)†	Replication study (n = 3,963)†
Women	66.9	66.0	67.0
Age, mean ± SD years	53.3 ± 13.4	52.0 ± 14.1	53.7 ± 13.5
ANA titer ≥1:40			
All	45.2	48.4	42.5
Speckled	43.7	46.8	41.1
Homogeneous	25.3	29.0	21.3
Nucleolar	4.7	5.1	4.2
Discrete speckled	0.9	0.8	0.9
Cytoplasmic	2.0	1.6	2.3

* Except where indicated otherwise, values are the percent. ANA = antinuclear antibody.
† In the genome-wide association study (GWAS), DNA samples were genome-scanned using Illumina HumanHap610, HumanHapOmni2.5-4, or HumanHapOmni2.5-8 arrays. Genotyping in the replication study was performed using a TaqMan Assay.

Next, we performed a GWAS for ANA positivity. A total of 3,185 participants and 303,506 markers that had passed criteria of inclusion and quality control were used for logistic regression analysis, with age and sex as covariates. As a result, the Q–Q plot indicated an inflation factor of 1.02, suggesting that the current study was free from population stratification (Figure 1). A significant association of rs9405108 in the HLA locus was observed at a P value of 8.9×10^{-8}. Conditioning rs9405108 to detect further associated markers in this region did not result in any markers showing significant associations ($P > 1.0 \times 10^{-4}$) (data not shown). No SNPs in non-HLA regions displayed suggestive associations ($P > 1.0 \times 10^{-5}$). We performed a replication study for rs9405108 using 3,963 participants (Table 1). For technical reasons, SNP rs2395185, which is almost in complete LD with rs9405108 ($D' = 1$ and $r^2 = 0.999$), was genotyped instead of rs9405108. As a result, the

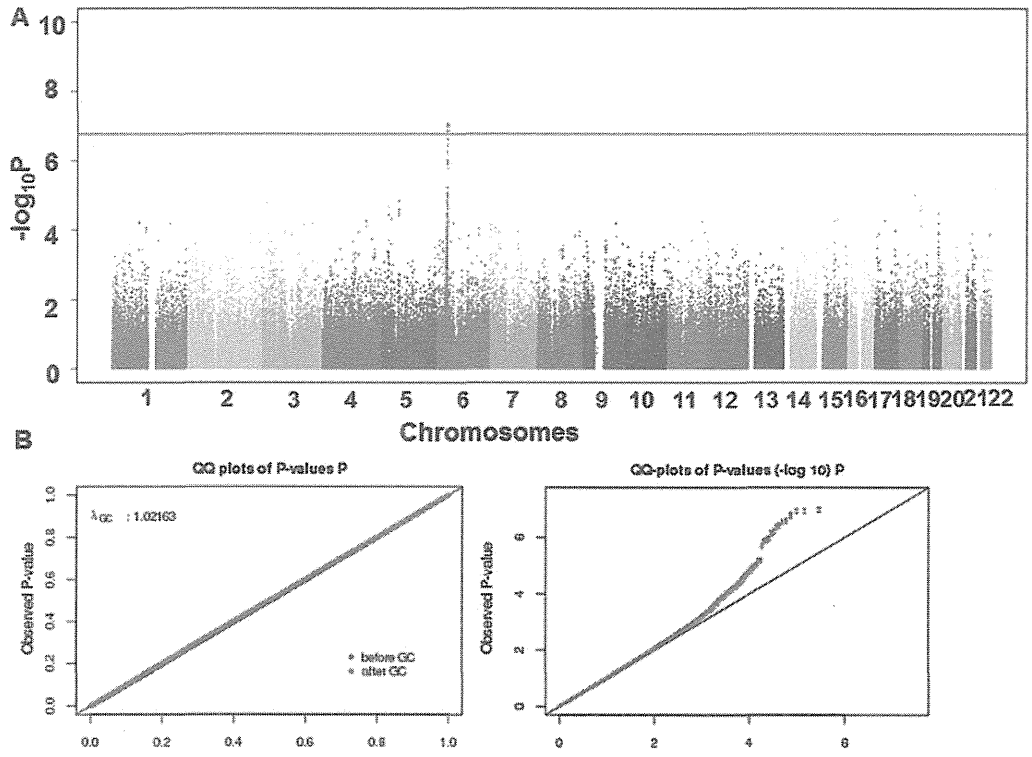

Figure 1. Genome-wide association study (GWAS) results for antinuclear antibody (ANA) production. **A,** Manhattan plot. The horizontal line indicates the significance level of the GWAS based on Bonferroni correction. **B,** Q–Q plots. λ_{gc} = genomic control inflation factor.

association of rs2395185 was replicated (overall $P = 1.3 \times 10^{-11}$) (Table 2).

SNP rs2395185 is located between the *HLA–DRA* and *HLA–DRB5* genes and is in moderate LD with HLA–DRB1*0405 ($r^2 = 0.42$). Considering that major histocompatibility complex proteins are respon-

Table 2. Associations of top SNPs with ANAs or their staining patterns*

SNP	Chr.	Position	ANA staining pattern	Nearest gene	Ref/var	Study	β	SE	OR (95% CI)	P
rs2395185	6	32541145	ANA (total)	*HLA–DRA*	G/T	GWAS	0.29	0.055	1.33 (1.20–1.48)	1.4×10^{-7}
						Replication	0.22	0.050	1.24 (1.12–1.37)	1.3×10^{-5}
						Overall	0.25	0.037	1.28 (1.19–1.38)	1.3×10^{-11}
rs2395185	6	32541145	Speckled	*HLA–DRA*	G/T	GWAS	0.29	0.055	1.33 (1.20–1.48)	1.4×10^{-7}
						Replication	0.22	0.050	1.25 (1.13–1.37)	8.3×10^{-6}
						Overall	0.25	0.037	1.29 (1.20–1.38)	7.5×10^{-12}
rs2395185	6	32541145	Homogeneous	*HLA–DRA*	G/T	GWAS	0.31	0.058	1.37 (1.22–1.54)	7.0×10^{-8}
						Replication	0.24	0.058	1.27 (1.13–1.42)	4.6×10^{-5}
						Overall	0.28	0.041	1.32 (1.22–1.43)	2.2×10^{-11}
rs6457300	6	31106721	Nucleolar	*C6orf205*	T/G	GWAS	−0.53	0.12	0.59 (0.46–0.74)	1.2×10^{-5}
						Replication	−0.13	0.11	0.88 (0.70–1.10)	0.26
						Overall	−0.32	0.083	0.73 (0.62–0.86)	0.00013
rs1611185	6	29876323	Discrete speckled	*HLA–G*	T/C	GWAS	1.28	0.29	3.61 (2.03–6.41)	1.2×10^{-5}
						Replication	0.19	0.25	1.21 (0.74–1.99)	0.44
						Overall	0.66	0.19	1.93 (1.32–2.80)	0.00060

* SNP = single-nucleotide polymorphism; ANAs = antinuclear antibodies; Chr. = chromosome; Ref/var = reference allele/variant allele; OR = odds ratio; 95% CI = 95% confidence interval; GWAS = genome-wide association study.

Table 3. Associations of SLE-related SNPs with ANA positivity and SLE susceptibility*

SNP	Chr.	Position	Gene	Ref/var	*P*	ANA OR (95% CI)†	SLE OR (95% CI)‡
Previous loci in Japanese population							
rs10168266	2	191644049	*STAT4*	T/C	0.20	1.08 (0.96–1.2)	1.59 (1.42–1.78)
rs340630	4	88177419	*AFF1*	A/G	0.13	1.08 (0.98–1.2)	1.21 (1.14–1.30)
rs9501626	6	32508322	*HLA*	A/C	0.62	1.04 (0.89–1.22)	1.86 (1.62–2.13)
rs2230926	6	138237759	*TNFAIP3*	G/T	0.15	1.16 (0.95–1.41)	1.75 (1.47–2.08)
rs6964720	7	75018280	*HIP1*	G/A	0.69	0.98 (0.86–1.1)	1.43 (1.27–1.63)
rs2254546	8	11381089	*BLK*	G/A	0.90	1.01 (0.9–1.13)	1.42 (1.25–1.61)
rs6590330	11	127816269	*ETS1*	A/G	0.015	1.14 (1.03–1.27)	1.44 (1.30–1.60)
Independent susceptibility SNPs of HLA locus in European population							
rs9265604	6	31407429	*HLA-B*	C/T	0.78	1.02 (0.92–1.13)	0.83 (0.78–0.89)
rs9378200	6	31680906	*BAT2*	C/T	0.17	0.92 (0.82–1.04)	0.59 (0.52–0.67)
rs9271731	6	32701590	*HLA-DRB1–HLA-DQA1*	G/A	0.41	1.06 (0.92–1.22)	1.34 (1.25–1.45)
rs9469220	6	32766288	*HLA-DQA1*	A/G	0.027	0.88 (0.78–0.98)	0.65 (0.61–0.68)

* SLE = systemic lupus erythematosus (see Table 2 for other definitions).
† For ANA positivity.
‡ For SLE susceptibility.

sible for self recognition and antigen presentation, the association between the polymorphisms in the HLA locus and ANAs seemed reasonable. HLA–DRB1*0405 is associated with a wide range of rheumatic and autoimmune diseases (26,31). This raised the possibility that autoimmune-related markers also had effects on ANA production. We selected SLE as being representative of autoimmune diseases with ANA production, and we analyzed the effects of a total of 7 markers that were reported to be associated with SLE in a previous Japa-

Table 4. Associations of ANA positivity with imputed HLA–DRB1 and HLA–DQB1 alleles*

HLA allele	Model	*P*	Corrected *P*†	OR (95% CI)	Accuracy
HLA–DRB1					
DRB1*0405	Dominant	3.0×10^{-5}	0.00081	1.43 (1.21–1.70)	0.902
DRB1*1302	Additive	3.6×10^{-5}	0.00097	0.69 (0.58–0.82)	0.997
DRB1*1201	Additive	0.00021	0.0057	0.58 (0.44–0.78)	0.704
DRB1*1401	Additive	0.069	1	0.80 (0.62–1.02)	0.746
DRB1*1101	Additive	0.095	1	0.77 (0.57–1.05)	0.827
DRB1*0901	Additive	0.11	1	1.13 (0.97–1.31)	1
DRB1*0701	Additive	0.23	1	0.37 (0.07–1.89)	1
DRB1*0803	Additive	0.33	1	1.10 (0.91–1.33)	0.987
DRB1*1502	Additive	0.52	1	0.95 (0.82–1.11)	0.998
DRB1*0401	Dominant	0.58	1	1.12 (0.74–1.71)	0.883
DRB1*1501	Additive	0.66	1	1.05 (0.86–1.28)	0.992
DRB1*1001	Additive	0.67	1	0.86 (0.42–1.74)	0.909
DRB1*1202	Additive	0.69	1	0.93 (0.64–1.34)	0.964
DRB1*0802	Additive	0.74	1	1.05 (0.77–1.45)	0.808
DRB1*0101	Dominant	0.90	1	1.01 (0.82–1.25)	0.992
HLA–DQB1					
DQB1*0301	Additive	3.5×10^{-5}	0.00095	0.71 (0.61–0.84)	0.888
DQB1*0604	Additive	0.00027	0.0073	0.71 (0.60–0.86)	1
DQB1*0401	Dominant	0.00031	0.0084	1.38 (1.16–1.65)	0.902
DQB1*0302	Dominant	0.0087	0.24	1.30 (1.07–1.59)	1
DQB1*0503	Additive	0.087	1	0.78 (0.58–1.04)	1
DQB1*0303	Additive	0.11	1	1.13 (0.97–1.31)	0.819
DQB1*0201	Dominant	0.15	1	3.46 (0.65–18.39)	1
DQB1*0402	Additive	0.20	1	1.18 (0.92–1.51)	0.907
DQB1*0602	Additive	0.49	1	1.08 (0.87–1.32)	1
DQB1*0601	Dominant	0.67	1	0.97 (0.83–1.12)	1
DQB1*0502	Dominant	0.75	1	0.95 (0.67–1.34)	1
DQB1*0501	Dominant	0.89	1	1.01 (0.83–1.24)	1

* See Table 2 for definitions.
† Corrected by Bonferroni adjustment.

nese study (15). The genotypes of these 7 markers were imputed using subjects in the Nagahama Study genotyped by denser arrays as a reference. All of the alleles showed good quality of imputation ($R^2 > 0.95$), but none of them displayed strong associations with ANA positivity ($P > 0.01$) (Table 3).

Since the HLA locus, especially HLA–DRB1, is the established locus for susceptibility to SLE with multiple independent associations shown beyond ethnicity (15,28,32), we analyzed detailed associations between the HLA locus and ANA positivity. A previous European study identified 5 independent SNPs that confer susceptibility to SLE (28). Because 1 of the 5 SNPs (rs1150703) is monomorphic in Japanese, the results for 4 SNPs are given in the current study (Table 3). None of them showed comparable associations in Europeans. We also performed imputation of HLA–DRB1 and HLA–DQB1 alleles (see Subjects and Methods). While the previous European study suggested the independent association of HLA–DQA1*0102 with SLE, we used HLA–DRB1*1501 and *1302 instead, which explained large parts of the association between HLA–DQA1*0102 and SLE (28). HLA–DRB1*0405, which was moderately tagged by rs2395185, showed a positive association with the smallest P value ($P_{corr} = 0.00081$) (Table 4). HLA–DQB1*0401 also showed a positive association, and HLA–DRB1*1302 and *1201 and HLA–DQB1*0301 and *0604 showed negative associations ($P_{corr} \leq 0.0084$) (Table 4). The associations of HLA–DQB1*0401, *0604, and *0301 seemed to be explained with HLA–DRB1*0405, HLA–DRB1*1302, and a combination of HLA–DRB1*1201 and HLA–DRB1*1101, respectively (r^2 values of 0.99, 0.92, and 0.59, respectively). HLA–DRB1*1501, the strongest susceptibility allele in Japanese (32), did not show a significant association (Table 4).

Considering the negative association of HLA–DRB1*1302 and the lack of association of HLA–DRB1*1501, HLA–DQA1*0102 was assumed to display a suggestive negative association. HLA–DRB1*0901, *0802, and *0401, which showed independent significant positive associations with SLE in Japanese (32), were not associated with ANA positivity.

Next, we addressed the similarities and differences of associations in the HLA locus among ANA staining patterns. Among the 2,820 SNPs in the HLA locus, rs9368726 and rs1964995, both of which were in strong LD with rs2395185 (r^2 values of 1.0 and 0.72, respectively), showed the strongest associations with speckled and homogeneous patterns, respectively ($P = 1.1 \times 10^{-7}$ and $P = 3.6 \times 10^{-8}$, respectively, in the GWAS) (Figure 2A). When we used the genotyping

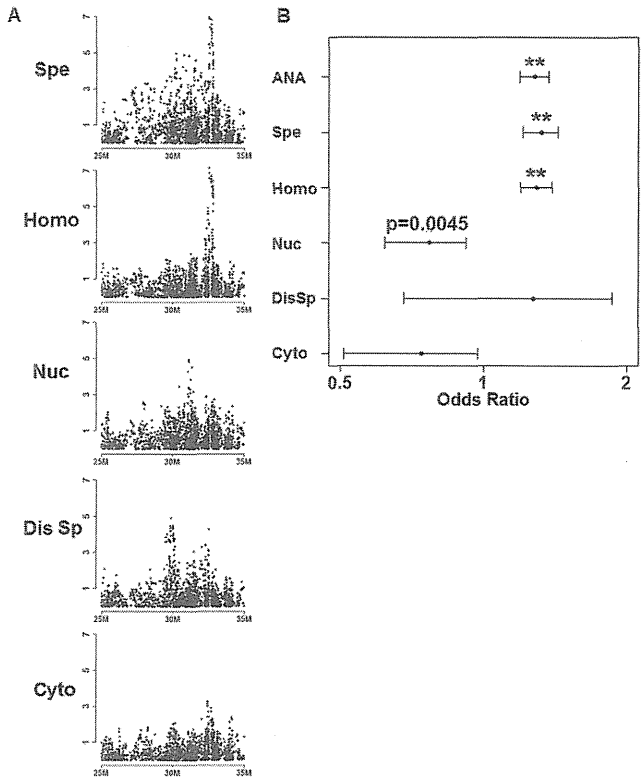

Figure 2. Heterogeneous association of the HLA locus among staining patterns of antinuclear antibodies (ANAs). **A**, Regional Manhattan plots for different staining patterns in the HLA region. **B**, Odds ratios and 95% confidence intervals of associations between rs2395185 and ANAs or their staining patterns. ** = $P < 1.0 \times 10^{-10}$. Spe = speckled; Homo = homogeneous; Nuc = nucleolar; Dis Sp = discrete speckled; Cyto = cytoplasmic.

results of rs2395185 instead of the 2 SNPs, the associations were also observed in the replication study (overall $P = 7.5 \times 10^{-12}$ and overall $P = 2.2 \times 10^{-11}$ for speckled and homogeneous patterns, respectively) (Table 2). The strongest associations with nucleolar and discrete speckled patterns in the HLA locus were observed for rs6457300 and rs1611185, respectively (both $P = 1.2 \times 10^{-5}$) (Table 2). Both SNPs are located >1.4 Mb from rs2395185. The cytoplasmic pattern showed the strongest association with rs9268347 ($P = 0.00052$), which is located 101 kb from rs2395185. We further genotyped rs6457300 and rs1611185 in the replication study, but the associations were not replicated (Table 2).

We focused on rs2395185 since it was the only SNP that demonstrated increasing effects on speckled and homogeneous patterns beyond levels significant in GWAS. Despite its increasing effects on the production of speckled and homogeneous patterns, the SNP displayed a significant decreasing effect on the nucleolar

pattern ($P = 0.0045$) (Figure 2B). Next, we analyzed whether rs2395185 had increasing effects on ANA levels in subjects positive for ANAs. When we examined subjects with ANA titers ≥1:40 and reviewed the staining patterns, the T allele of rs2395185 showed suggestive or significant increasing effects on levels of total, speckled, and homogeneous patterns ($P = 0.12$, $P = 0.016$, and $P = 0.00030$, respectively, by Jonckheere-Terpstra test).

DISCUSSION

The current study provided solid evidence of the distribution and correlates of ANAs in a Japanese adult population. This is the first study to perform GWAS of ANAs in healthy populations and detect a significant locus. The nucleolar pattern has characteristics that differ from those of other staining patterns. Autoantibodies such as anti–U3 RNP, anti-Th/To, or antiribosomal antibodies, associated with systemic sclerosis or SLE, are classified as having the nucleolar pattern of ANAs.

In our study, 12.5% of healthy participants had ANA titers of ≥1:80, which is comparable to previous results in the US (4,754 individuals, 13.8%) (14). The percentages were slightly higher than in previous studies for the cutoff level of 1:40 and comparable for the cutoff level of 1:160 (~26.8–31.7% and ~5.0–8.1%, respectively, in previous studies). Of the 201 subjects who were excluded due to the possibility of having autoimmune diseases, 141 had ANA titers of ≥1:40 (70.1%) (data not shown), suggesting the validity of the exclusion criteria. The increase in ANA positivity in women was confirmed, and this association could partly be explained by sex hormones (33–35). Considering the sex difference in onset of autoimmune diseases, the same undetermined mechanisms related to sex may underlie ANA production in healthy populations.

This study showed a strong effect of age on positivity for ANAs. We did not observe an increase in positivity for ANAs with aging in subjects 30–50 years old ($P = 0.20$) (data not shown); therefore, the elderly populations largely accounted for the association between aging and ANA positivity. The increase in ANAs after age 50 years matches the results in the US study. This association might be explained by dysregulation of immunologic tolerance in the elderly population. Considering the previous reports of high ANA levels in the adolescent population (13,36), the association between ANA positivity and aging in the general population seems to have a "U" pattern (lowest ANA levels at ages with most frequent reproduction). The effects of age and sex on ANAs seemed to differ among the staining patterns. The nucleolar pattern did not display significant associations with age and sex. As discrete speckled patterns showed positive associations, the lack of association of the nucleolar pattern with age and sex cannot be explained by its frequency.

Correlates of ANAs seemed to partly differ between different populations. The current study did not find a significant association between obesity and ANA positivity. However, obesity tended to be inversely related to ANA positivity as in the US study, and the limited number of obese individuals in the current study might explain this nonsignificant association. The association between increased CRP levels and ANA positivity was not found in the previous study. Chronic mild inflammation would lead to the production of ANAs. Since the distribution of CRP levels in subjects differs greatly between the 2 studies, further analysis would clarify the association.

The current study identified rs2395185 in the HLA class II locus as a marker of susceptibility to ANA positivity. It should be noted that a previous study showed an association between rs2395185 and ulcerative colitis (37), suggesting the involvement of rs2395185 with autoimmune processes. Because a previous study showed that the type I interferon (IFN) signature is up-regulated in healthy populations with high ANA titers (38), it will be interesting to analyze the functional roles of rs2395185 in the type I IFN pathway.

The T allele of rs2395185 showed increasing effects on levels of speckled and homogeneous patterns, but a decreasing effect on levels of the nucleolar pattern. This indicates that the nucleolar pattern also differs from the speckled and homogeneous patterns in terms of HLA association. The detailed plots in the HLA locus support the notion of different association patterns among ANA staining patterns. The opposing effect of rs2395185 on levels of the nucleolar pattern indicates that the lack of common association of rs2395185 over staining patterns of ANAs was not due to lower positivity for several staining patterns. As the HLA class II locus is strongly associated with presentation and recognition of antigen, the current results may suggest that ANA production is associated with binding affinity of antigens to the HLA molecule. Since antigens recognized by ANAs contain a wide variety of molecules, the common strong association of 1 polymorphism with speckled and homogeneous patterns suggests similarity or cross-reactivity of antigens that correspond to speckled or homogeneous patterns. The opposing effect also suggests that antigens corresponding to the nucleolar pattern are not presented by common HLA class II alleles with speckled and homogeneous patterns.

As HLA–DRB1*0405 is associated with susceptibility to immunologic disorders or autoantibody production in autoimmune diseases (27,39), the association between ANA production and rs2395185 in LD with HLA–DRB1*0405 might suggest a common mechanism between HLA–DRB1*0405–related autoimmune disease susceptibility and production of ANAs. At the same time, the association raises the possibility that genes conferring susceptibility to ANA positivity might be identified as genes conferring susceptibility to connective tissue diseases.

However, the current study did not detect significant associations between SLE-related SNPs or HLA alleles and ANA positivity. These results indicated that SNPs significantly associated with SLE in the previous study were associated with SLE itself and not with ANAs. Lack of association between ANA production in healthy subjects and rs9501626 or HLA–DRB1*1501, the most significant HLA SNP or HLA–DRB1 allele associated with SLE in the Japanese population, may suggest that autoantigens recognized by ANAs in SLE patients are different from those recognized by ANAs in healthy populations. In fact, a previous study showed that healthy subjects with high ANA titers exhibited an autoantibody profile distinct from that in SLE patients (38). These results may also suggest the involvement of immunologic molecular pathways in SLE development that are not related to ANA production in healthy populations. While we did not find associations of the 7 SNPs in Japanese and the 4 SNPs in Europeans, we observed that 9 of the 11 SNPs had a common direction of association between SLE susceptibility and ANA positivity. All the susceptibility DRB1 alleles in Japanese (HLA–DRB1*1501, *0901, *0802, and *0401) also showed a trend toward increasing ANAs. The common directionality between SLE susceptibility and ANA positivity may be meaningful.

It will be interesting to finely genotype the HLA locus to determine the polymorphisms and mechanisms responsible for causing the associations with ANAs or speckled and homogeneous patterns. None of the polymorphisms display significant associations with nucleolar, discrete speckled, or cytoplasmic patterns. However, considering the low positivity for these staining patterns and the strength of associations in the HLA locus in the current study, increasing the number of subjects would identify yet-to-be-determined polymorphisms associated with these staining patterns. We did not observe significant associations with ANA positivity outside the HLA locus. In addition, none of the polymorphisms outside the HLA locus showed suggestive associations with ANA staining patterns (data not shown). The significance and roles of ANAs in healthy populations have not yet been clarified. Because a previous study showed that the type I IFN signature is up-regulated in healthy populations with high ANA titers (38), it is possible that high ANA titers in healthy populations reflect a pre–autoimmune disease state. Further followup and analyses are necessary to address these points.

Taken together, the current study determined that the HLA class II locus is a locus for susceptibility to ANA production. Genetic overlap between SLE susceptibility and ANA production in healthy populations is limited. The current results indicate that ANAs are not homogeneous autoantibodies with similar characteristics. It is feasible to analyze whether the current results are observed in different populations, especially in Europeans.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Terao had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Analysis and interpretation of data. Terao, Kawaguchi.

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BRIEF REPORT

A detection algorithm for drug-induced liver injury in medical information databases using the Japanese diagnostic scale and its comparison with the Council for International Organizations of Medical Sciences/the Roussel Uclaf Causality Assessment Method scale

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ABSTRACT

Purpose Drug-induced liver injury (DILI) is one of the primary targets for pharmacovigilance using medical information databases (MIDs). Because of diagnostic complexity, a standardized method for identifying DILI using MIDs has not yet been established. We applied the Digestive Disease Week Japan 2004 (DDW-J) scale, a Japanese clinical diagnostic criteria for DILI, to a DILI detection algorithm, and compared it with the Council for International Organizations of Medical Sciences/the Roussel Uclaf Causality Assessment Method (CIOMS/RUCAM) scale to confirm its consistency. Characteristics of DILI cases identified by the DDW-J algorithm were examined in two Japanese MIDs.

Methods Using an MID from the Hamamatsu University Hospital, we constructed a DILI detection algorithm on the basis of the DDW-J scale. We then compared the findings between the DDW-J and CIOMS/RUCAM scales. We examined the characteristics of DILI after antibiotic treatment in the Hamamatsu population and a second population that included data from 124 hospitals, which was derived from an MID from the Medical Data Vision Co., Ltd. We performed a multivariate logistic regression analysis to assess the possible DILI risk factors.

Results The concordance rate was 79.4% between DILI patients identified by the DDW-J and CIOMS/RUCAM; the Spearman rank correlation coefficient was 0.952 ($P < 0.0001$). Men showed a significantly higher risk for DILI after antibiotic treatments in both MID populations.

Conclusions The DDW-J and CIOMS/RUCAM algorithms were equivalent for identifying the DILI cases, confirming the utility of our DILI detection method using MIDs. This study provides evidence supporting the use of MID analyses to improve pharmacovigilance.

KEY WORDS—drug-induced liver injury; medical information database; pharmacovigilance; DDW-J; antibiotics; pharmacoepidemiology

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INTRODUCTION

Drug-induced liver injury (DILI) is a clinically problematic issue and a major cause of acute liver failure.[1–3] In general, DILI diagnosis is complex and nonstandardized because of the difficulty in detection and lack of reliable markers.[4,5] Therefore, clinical scales were developed to facilitate DILI diagnosis. The Council for International Organizations of Medical Sciences/the Roussel Uclaf Causality Assessment Method (CIOMS/RUCAM) scale was proposed[6] and has been generally used as a standardized diagnostic tool. In Japan, the Digestive Disease Week Japan 2004 (DDW-J) scale, which is highly sensitive (92.1%) and specific (88.1%), was developed by modifying the CIOMS/RUCAM scale.[7,8] In particular, the factor of co-medication was excluded, and the factors of drug lymphocyte stimulation test and eosinophilia were included according to Japan's clinical environment.

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Challenges using medical information databases (MIDs) for identifying DILI have been addressed worldwide,[9,10] but a standardized method for such analyses has not yet been established. Because a diagnosis scale based on numerical or quantitative information was considered suitable for MID-based research, we constructed a detection algorithm for DILI on the basis of the DDW-J scale.

METHODS

Data source and ethics

This study was performed using two data sources: one was a high-speed retrieval system at the Hamamatsu University Hospital (Shizuoka, Japan),[11] and the other was a commercial MID developed by the Medical Data Vision Co., Ltd. (MDV, Tokyo, Japan) that contained data from 124 large and mainly tertiary hospitals in Japan. The mean follow-up period within this MID was 243 days. MIDs from Hamamatsu and MDV included health records from approximately 200 000 and 4 400 000 patients, respectively. The two MIDs had similar age structures. We used only anonymized data in our analysis. This study was approved by the ethics committees of both the Hamamatsu University School of Medicine and the National Institute of Health Sciences.

Study population

Clarithromycin (CM), azithromycin (AM), levofloxacin (LX), and moxifloxacin (MX) for internal use were examined in this study because of their similar clinical indications and their wide use in Japan. The subject inclusion criteria were as follows: (i) received at least one prescription for one of the study drugs between 1 April 2007 and 31 March 2012 in the Hamamatsu MID and between 1 April 2008 and 31 August 2011 in the MDV MID; (ii) no other study drug prescription between 90 days prior to the index date (the first day of the study drug administration) and the last administration in the first prescription term (>180-day interval between the study drug administrations); (iii) 18 years old or older at the index date; (iv) received alanine aminotransferase, and alkaline phosphatase tests in the preceding period (within 90 days prior to the index date) and the follow-up period (within 180 days after the last administration); (v) no occurrence of liver injury (alanine aminotransferase > 2 × the upper limit of normal value or alkaline phosphatase > upper limit of normal value) in the preceding period; (vi) no medical history in the preceding period of HIV (B20-24) or cancer (C00-97) as determined by the International Statistical Classification of Diseases and Related Health Problems, 10th revision.

Characteristics

On the basis of the general considerations for usage and dosage in each label, a long treatment was considered ≥8 days with CM, LX, and MX and ≥4 days with AM; a high dose was considered an average daily dose of >400 mg/day for CM and MX, >500 mg/day for AM and LX, and >2000 mg/day for AM in dry syrup form for single administration.

Algorithm for identifying drug-induced liver injury

We applied the original DDW-J scoring to the DILI detection algorithm consistently.[12] In addition, the algorithm based on the CIOMS/RUCAM scale was used as a reference.[6] Details regarding these two scales are summarized in Table S1. According to the definition of each scale, DILI was defined as a total score ≥5 in the DDW-J and ≥6 in the CIOMS/RUCAM algorithm.

Statistical analysis

To calculate the odds ratios (ORs) for DILI onset and those between DILI and non-DILI groups, we performed a multivariate logistic regression analysis adjusting for age (≥55 years), gender, in/outpatient status, diabetes mellitus, treatment duration, and high dose. Values of $P < 0.05$ (two-sided) were considered statistically significant. All statistical analyses were conducted using SAS software, version 9.3 (SAS Institute Inc., NC, USA).

RESULTS

Assessment of drug-induced liver injury detection algorithm

Using the DDW-J algorithm in the Hamamatsu population, we detected 182 DILI patients. To assess the utility of the DDW-J algorithm, we compared the results with those obtained with the CIOMS/RUCAM algorithm. Because the CIOMS/RUCAM scale excludes the delayed onset cases (>15 days for hepatocellular type or >30 days for cholestatic or mixed type after stopping the drug) from scoring except when dealing with slowly metabolized chemicals, the comparison was performed in the nondelayed onset population (Figure 1). The concordance rate for DILI patients between the two algorithms was 79.4%; the Spearman rank correlation coefficient was 0.952 ($P < 0.0001$). Although the CIOMS/RUCAM scale does not explicitly define slowly metabolized chemicals, AM has a longer half-life than other drugs.

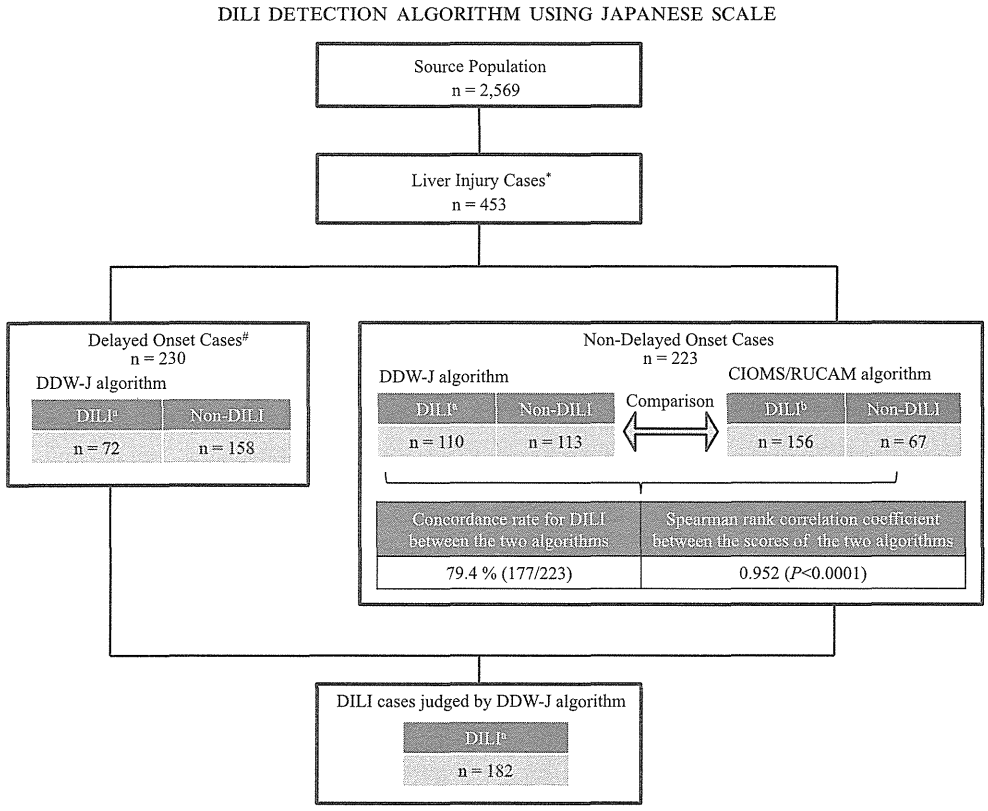

Figure 1. Identification of drug-induced liver injury (DILI) cases in the Hamamatsu population. *Patients with alanine aminotransferase > 2 × the upper limit of normal value (ULN) or alkaline phosphatase >ULN from the index date to 180 days after the last administration. #Patients in which the liver injury occurred after 15 days for the hepatocellular type, or more than 30 days for the cholestatic or mixed type, following the last administration. [a]Defined as a total score ≥5 in the Digestive Disease Week Japan 2004 (DDW-J) algorithm. [b]Defined as a total score ≥6 in the Council for International Organizations of Medical Sciences/the Roussel Uclaf Causality Assessment Method (CIOMS/RUCAM) algorithm

We therefore performed sensitivity analysis that incorporated the delayed onset cases prescribed AM into the comparison. This analysis showed consistent findings with a concordance rate of 78.4%.

Presence of possible alternative causes in DILI cases was compared with liver injury cases judged as non-DILI by the DDW-J algorithm in the Hamamatsu population (Table S2). The results showed that patients with alternative causes, such as viral hepatitis, were effectively excluded by this algorithm.

Characteristics of drug-induced liver injury patients

The study population sizes in MIDs from Hamamatsu and MDV were 2569 and 3856, respectively. To examine the characteristic of DILI patients, the ORs for DILI identified by the DDW-J algorithm were calculated (Table 1, with details in Table S3). The ORs of DILI onset in men were 1.44 (95% confidence interval, 1.05–1.98) in the Hamamatsu MID and 1.32 (95% confidence interval, 1.01–1.72) in the MDV MID. Because there were considerable differences in the average treatment duration, we performed an additional sub-analysis on treatment duration stratified by the study drugs. In the MDV MID, CM and LV subpopulations showed a significant association between a long treatment duration and DILI.

DISCUSSION

We demonstrated that the DDW-J algorithm was highly compatible with the CIOMS/RUCAM algorithm in the Hamamatsu MID (Figure 1). This indicates the DDW-J algorithm has adequate generalizability in assessing DILI. Using the DDW-J algorithm, we examined the characteristics of DILI cases by assessing the potential risk factors. Furthermore, we used the same study protocol to investigate a second population that included patients from multiple hospitals (MDV MID) to improve the robustness of our results. As a result, men showed a significantly higher risk for DILI in both populations. This finding is inconsistent with those of previous reports, although the role of gender in DILI remains controversial.[4] Alcohol consumption is one of the criteria in both the DDW-J and CIOMS/RUCAM scales, but this information was not available in the

Table 1. Comparison of odds ratios (ORs) for onset of drug-induced liver injury (DILI) in two medical information databases (MIDs)

	Hamamatsu University Hospital MID				MDV MID			
Characteristics	*n*	OR*	95% CI	*P*-value	*n*	OR*	95% CI	*P*-value
Total	2569				3856			
Age ≥55 years		1.49	1.02–2.17	0.0371		0.85	0.63–1.16	0.3052
Male		1.44	1.05–1.98	0.0237		1.32	1.01–1.72	0.0409
Inpatient		1.38	1.01–1.90	0.0452		1.30	0.99–1.72	0.0624
Diabetes mellitus		0.81	0.47–1.38	0.4316		0.90	0.60–1.36	0.6225
Long treatment[a]		1.14	0.83–1.57	0.4225		1.46	1.10–1.94	0.0082
High dose[b]		1.83	0.81–4.16	0.1473		1.34	0.73–2.48	0.3436
Clarithromycin sub-group	524				845			
Days ≥8		1.19	0.56–2.52	0.6531		3.18	1.59–6.37	0.0011
Days ≥28		2.08	0.91–4.80	0.0846		2.97	1.43–6.15	0.0034
Levofloxacin sub-group	1551				2441			
Days ≥8		1.15	0.75–1.76	0.5273		1.57	1.10–2.23	0.0122

CI, confidence interval; DILI, defined as Digestive Disease Week Japan 2004 score ≥5.
[a]Patients whose treatment duration was ≥8 days in clarithromycin, levofloxacin, and moxifloxacin and ≥4 days in azithromycin.
[b]Patients whose average dose was beyond the usual approved dose.
*Adjusted for age (≥55 years), gender, in/outpatient status, diabetes mellitus, treatment duration, and high dose.

current study. Because the national survey in Japan indicated that alcohol consumption was remarkably higher in men than in women (35.1% vs. 7.7%),[13] the gender difference in alcohol consumption might have led to a higher risk in men.

Regarding treatment duration, a longer treatment with CM and LX, which included an adequate population size in this study, was significantly associated with DILI in the MDV population. In the Hamamatsu population, the long treatment groups, especially the ≥28-day CM group, showed a tendency toward a higher risk for DILI, although the associations were not significant. These results might indicate that DILI should be carefully monitored during the long-term treatments with antibiotics. Although further confirmation in a larger-scale study is necessary, our algorithm, which is based on a clinical diagnostic scale, could be a useful method to identify DILI and access its risk-related information through MID research.

The current study has some limitations. The DDW-J and CIOMS/RUCAM scoring systems were designed for prospective diagnoses of individual cases, and their utilities in retrospective studies, including the quality of DILI cases identified by our algorithms, were not validated. Furthermore, we could not retrieve additional information from the MIDs used in this study, such as drinking habits and pregnancy, which constitutes parts of the scoring systems. This might lead to underestimation of DILI risk. In addition, articles on the DDW-J were predominantly published in Japanese-language journals, which makes it difficult for non-Japanese researchers to assess and utilize the DDW-J scale. Although regional DILI scoring would still be required for diagnostic purpose when considering the Japanese medical environment, the adoption of a uniform diagnostic approach will be preferable in future.

In conclusion, we have proposed a useful method that uses MIDs for identifying DILI. Our study supports the utility of MID research in pharmacovigilance.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

KEY POINTS
- A standardized detection method for DILI using MIDs has not yet been established because of the complexity of diagnosis.
- We applied a Japanese DILI diagnostic scale, DDW-J, to a DILI detection algorithm that is applicable for assessment of potential risk factors.
- The DDW-J algorithm was compatible with the international CIOMS/RUCAM scale, which indicates the utility of the algorithm.
- This study supports the utility of MID-based research for improving pharmacovigilance.

ETHICS STATEMENT

This study was approved, including procedures for informed consent, by the ethics committees of both the Hamamatsu University School of Medicine and the National Institute of Health Sciences.

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