研究代表者：松田　文彦

発表者名	演題名	学会名	会場	日時
松田　文彦	ゲノムワイド関連解析を用いた非アルコール性脂肪性肝疾患の関連遺伝子の探索	第48回日本肝臓学会	ポルテ金沢（金沢）	2012年6月7日
松田　文彦	分子を通して自分を知る未病社会の健康観～大規模コホート研究とゲノム、タンパク、代謝物～	未病社会の診断技術研究会第7回講演会	東京大学武田ホール（東京）	2012年10月11日
松田　文彦	Human Biology とゲノム情報	日本DNA多型学会第21回学術集会シンポジウム	京都教育文化センター（京都）	2012年11月7日
松田　文彦	ヒト生命情報統合研究とそのモデルケースとしてのながはまゲノムコホート事業	第59回日本臨床検査医学会学術集会シンポジウム	国立京都国際会館（京都）	2012年11月30日

研究分担者：辻　省次

発表者名	演題名	学会名	会場	日時
三井　純、後藤　順、辻　省次	エクソーム解析による遺伝的異質性の高い疾患に対する遺伝子検査	第20回日本遺伝子診療学会	アクトシティ浜松（浜松）	2013年7月19日

研究分担者：松原　洋一

発表者名	演題名	学会名	会場	日時
飯倉　立夏、青木　洋子、新堀　哲也、小松崎　匠子、松原　洋一	東北大学病院遺伝科の現状	日本人類遺伝学会第57回大会	京王プラザホテル（東京）	2012年10月25日
齋藤　由佳、青木　洋子、村松　秀樹、今泉　益栄、力石　健、笹原　洋二、呉　繁夫、新堀　哲也、小島　勢二、松原　洋一	Noonan 症候群類縁疾患と小児血液腫瘍における CBL の分子遺伝学的解析	日本人類遺伝学会第57回大会	京王プラザホテル（東京）	2012年10月27日

研究分担者：松本　直通

発表者名	演題名	学会名	会場	日時
Matsumoto, N.	Exome analysis in mendelian disorders	2012 Illumina Asia Pacific Scientific Summit (invited)	Gold Coast, Austraria	April 24, 2012
Matsumoto, N.	Exome sequencing in mendelian disorders.	Translational Genomics Conference 2012 (invited)	Jeju, Korea	October 13, 2012
Matsumoto, N.	Medelian exome.	The 12th annual meeting of East Asian Union of Human Genetics Societies (invited)	Seoul, Korea	November 29, 2012

発表者名	演題名	学会名	会場	日時
松本　直通	小児神経疾患における遺伝子研究の新潮流	第 5 回みやこ小児神経臨床懇話会（招聘講演）	メルパルク 京 都（京都）	2012年6月9日
松本　直通	自閉症スペクトラムとてんかんに着目したゲノム解析	第 34 回日本生物学的精神医学会（招聘講演）	神戸国際会 議 場（神戸）	2012 年 9 月 28 日
松本　直通	遺伝性疾患のエクソーム解析	生命医薬情報学連合大会 2012（招聘講演）	タワーホール船堀（東京）	2012 年 10 月 17 日
松本　直通	Isolation of genes causative for genetic diseases by next generation sequencer.	日本人類遺伝学会第 57 回大会(Symposist)	京王プラザホテル（東京）	2012 年 10 月 25 日
松本　直通	次世代シーケンスを用いた疾患ゲノム解析	ゲノム解析懇話会（招聘講演）	京王プラザホテル（東京）	2012 年 10 月 25 日
松本　直通	エクソーム解析	第 152 回染色体研究会（招聘講演）	東京医科大学病院（東京）	2012 年 12 月 1 日
松本　直通	発達障害におけるゲノム解析：次世代技術を用いて	第 35 回日本分子生物学会年会（ワークショップ）	福岡国際会 議 場（福岡）	2012 年 12 月 13 日
松本　直通	遺伝性疾患のエクソーム解析	Advans 研 究 会 2012（招聘講演）	ホテルグランドパレス東京（東京）	2012 年 12 月 15 日

研究分担者：山田　亮

発表者名	演題名	学会名	会場	日時
Fujii, Y., Narita, T., Takeda, S., and Yamada, R.	Isotonic regression-based method for high throughput genotoxicity screenings.	Congress of the European Societies of Toxicology	Stockholm, Sweden	June 18, 2012.
Kajimoto, S. and Yamada, R.	An orthogonal matrix, which is useful to interpret the restriction of marginal counts of multi-way tables.	XXVIth International Biometric Conference	Kobe, Japan	August 27, 2012
Yamada, R.	Invited Session 3 : Statistical challenges in the analysis of rare genetic variants in association studies, discussing comment.	XXVIth International Biometric Conference	Kobe, Japan	August 27, 2012
Narahara, M., Tamaki, K., and Yamada, R.	DNA-based identifications in mass fatality incidents based on probabilities conditional on an entire dataset.	XXVIth International Biometric Conference	Kobe, Japan	August 28, 2012
Narahara, M., Nakayama, T., Matsuda, F., Nagahama Cohort Research Group, Maruyama, M., and Yamada, R.	動脈弾性指標における遺伝率推定	日本人類遺伝学会第 57 回大会	京王プラザホテル（東京）	2012 年 10 月 25 日

研究分担者：寺尾　知可史

発表者名	演題名	学会名	会場	日時
Terao, C., Ohmura, K., Ikari, K., Kochi, Y., Maruya, E., Katayama, M., Yurugi, K., Shimada, K., Murasawa, A., Honjo, S., Takasugi, K., Matsuo, K., Tajima, K., Suzuki, A., Yamamoto, K., Momohara, S., Yamanaka, H., Yamada, R., Saji, H., Matsuda, F. and Mimori,, T.	ACPA-negative rheumatoid arthritis consists of two genetically distinct subsets based on RF positivity.	EULAR（ヨーロッパリウマチ学会）2012	Berlin, Germany	June 9, 2012
Terao, C., Ohmura, K., Kawaguchi, Y., Nishimoto, T., Kawasaki, A., Takehara, K., Furukawa, H., Kochi, Y., Ota, Y., Ikari, K., Sato, S., Tohma, S., Yamada, R., Yamamoto, K., Kubo, M., Yamanaka, H., Kuwana, M., Tsuchiya, N., Matsuda, F. and Mimori, T.	HLA region conferring levels of anti-nuclear antibody in a Japanese population.	日本人類遺伝学会第 57 回大会	京王プラザホテル（東京）	2012 年 10 月 25 日
Terao, C., Hashimoto, M., Yamamoto, K., Murakami, K., Ohmura, K., Nakashima, R., Yamakawa, N., Yoshifuji, H., Yukawa N., Kawabata, D., Usui, T., Yoshitomi, H., Furu, M., Yamada, R., Matsuda, F., Ito, H., Fujii, T. and Mimori, T.	Three groups of joint synovitis in rheumatoid arthritis -analysis in KRAMA database-.	第 41 回日本免疫学会学術集会	神戸国際会議場（神戸）	2012 年 12 月 6 日

Ⅷ．研究成果による特許等の知的財産権の 出願・登録状況

研究分担者：松本　直通

種　類	受付（識別）番号	出願日
特許取得	PCT/JP2012/83113	2012 年 12 月 20 日
特許取得	PCT/JP2012/77903	2012 年 10 月 29 日
特許取得	特願 2012-180356	2012 年 8 月 16 日

IX. 研究成果の刊行物・別刷

論文リスト

1. Terao, C., Ohmura, K., Kawaguchi, Y., Nishimoto, T., Kawasaki, A., Takehara, K., Furukawa, H., Kochi, Y., Ota, Y., Ikari, K., Sato, S., Tohma, S., Yamada, R., Yamamoto, K., Kubo, M., Yamanaka, H., Kuwana, M., Tsuchiya, N., Matsuda, F. and Mimori, T. (2013) *PLD4* as a novel susceptibility gene for systemic sclerosis in a Japanese population. *Arthritis Rheum.* **65**, 472-480.

2. Terao, C., Ohmura, K., Ikari, K., Kochi, Y., Maruya, E., Katayama, M., Yurugi, K., Shimada, K., Murasawa, A., Honjo, S., Takasugi, K., Matsuo, K., Tajima, K., Suzuki, A., Yamamoto, K., Momohara, S., Yamanaka, H., Yamada, R., Saji, H., Matsuda, F. and Mimori, T. (2012) ACPA-negative RA consists of two genetically distinct subsets based on RF positivity in Japanese. *PLoS One.* **7**, e40067.

3. Kawaguchi, T., Sumida, Y., Umemura, A., Matsuo, K., Takahashi, M., Takamura, T., Yasui, K., Saibara, T., Hashimoto, E., Kawanaka, M., Watanabe, S., Kawata, S., Imai, Y., Kokubo, M., Shima, T., Park, H., Tanaka, H., Tajima, K., Yamada, R., Matsuda, F. and Okanoue, T. for the Japan Study Group of Nonalcoholic Fatty Liver Disease (JSG-NAFLD) (2012) Genetic polymorphisms of the human PNPLA3 gene are strongly associated with severity of non-alcoholic fatty liver disease in Japanese. *PLoS ONE* **7**, e38322.

4. Okada, Y., Terao, C., Ikari, K., Kochi, Y., Ohmura, K., Suzuki, A., Kawaguchi, T., Stahl, E.A., Kurreeman, F.A.S., Nishida, N., Ohmiya, H., Myouzen, K., Takahashi, M., Sawada, T., Nishioka, Y., Yukioka, M., Matsubara, T., Wakitani, S., Teshima, R., Tohma, S., Takasugi, K., Shimada, K., Murasawa, A., Honjo, S., Matsuo, K., Tanaka, H., Tajima, K., Suzuki, T., Iwamoto, T., Kawamura, Y., Tanii, H., Okazaki, Y., Sasaki, T., Gregersen, P.K., Padyukov, L., Worthington, J., Siminovitch, K.A., Lathrop, M., Taniguchi, A., Takahashi, A., Tokunaga, K., Kubo, M., Nakamura, Y., Kamatani, N., Mimori, T., Plenge, R.M., Yamanaka, H., Momohara, S., Yamada, R., Matsuda, F. and Yamamoto, K. (2012). Meta-analysis identifies nine new loci associated with rheumatoid arthritis in the Japanese population. *Nat. Genet.* **44**, 511–516.

5. Kato, L., Beguma, N. A., Burroughs, M., Doi, T., Kawai, J., Daub, C. O., Kawaguchi, T., <u>Matsuda, F.</u>, Hayashizaki, Y. and Honjo, T. (2012) Nonimmunoglobulin target loci of activation-induced cytidine deaminase (AID) share unique features with immunoglobulin genes. *Proc. Natl., Acad. Sci. U. S. A.* **109**, 2479-2484.

6. Okada, Y., Shimane, K., Kochi, Y., Tahira, T., Suzuki, A., Higasa, K., Takahashi, A., Horita, T., Atsumi, T., Ishii, T., Okamoto, A., Fujio, K., Hirakata, M., Amano, H., Kondo, Y., Ito, S., Takada, K., Mimori, A., Saito, K., Kamachi, M., Kawaguchi, Y., Ikari, K., Mohammed, O.W., Matsuda, K., Terao, C., Ohmura, K., Myouzen, K., Hosono, N., Tsunoda, T., Nishimoto, N., Mimori T., Matsuda, F., Tanaka, Y., Sumida, T., Yamanaka, H., Takasaki, Y., Koike, T., Horiuchi, T., Hayashi, K., Kubo, M., Kamatani, N., Yamada, R., Nakamura, Y. and Yamamoto, K. (2012) A genome-wide association study identified *AFF1* as a susceptibility locus for systemic lupus eyrthematosus in Japanese. *PLoS Genet.* **8**, e1002455.

7. Terao, C., Ikari, K., Ohmura, K., Suzuki, T., Iwamoto, T., Takasugi, K., Saji, H., Taniguchi, A., Momohara, S., Yamanaka, H., <u>Matsuda, F.</u> and Mimori, T. (2012) Quantitative effect of HLA-DRB1 alleles to ACPA levels in Japanese rheumatoid arthritis: no strong genetic impact of shared epitope to ACPA levels after stratification of HLA-DRB1*09:01. *Ann. Rheum. Dis.* **71**, 1095-1097.

8. Mitsui, J., Matsukawa, T., Ishiura, H., Higasa, K., Yoshimura, J., Saito, T. L., Ahsan, B., Takahashi, Y., Goto, J., Iwata, A., Niimi, Y., Riku, Y., Goto, Y., Mano, K., Yoshida, M., Morishita, S. and Tsuji S. (2012) *CSF1R* mutations identified in three families with autosomal dominantly inherited leukoencephalopathy. *Am. J. Med. Genet. B. Neuropsychiatr. Genet.* **159B**, 951-957.

9. Ishiura, H., Sako, W., Yoshida, M., Kawarai, T., Tanabe, O., Goto, J., Takahashi, Y., Date, H., Mitsui, J., Ahsan, B., Ichikawa, Y., Iwata, A., Yoshino, H., Izumi, Y., Fujita, K., Maeda, K., Goto, S., Koizumi, H., Morigaki, R., Ikemura, M., Yamauchi, N., Murayama, S., Nicholson, G.A., Ito, H., Sobue, G., Nakagawa, M., Kaji, R. and Tsuji, S. (2012) The TRK-fused gene is mutated in hereditary motor and sensory neuropathy with proximal dominant involvement. *Am. J. Hum. Genet.* **91**, 320-329.

10. Ishii, A., Saito, Y., Mitsui, J., Ishiura, H., Yoshimura, J., Arai, H., Yamashita, S., Kimura, S., Oguni, H.,

Morishita, S., Tsuji, S., Sasaki, M. and Hirose, S. Identification of *ATP1A3* mutations by exome sequencing as the cause of alternating hemiplegia of childhood in Japanese patients. (2012) *PLoS One.* 8, e56120.

11. Tsuji, S. The neurogenomics view of neurological diseases. (2013) *JAMA Neurol.* 9, 1-6.

12. Kikuchi, A., Arai-Ichinoi, N., Sakamoto, O., Matsubara, Y., Saheki, T., Kobayashi, K., Ohura, T. and Kure, S. (2012) Simple and rapid genetic testing for citrin deficiency by screening 11 prevalent mutations in *SLC25A13*. *Mol. Genet. Metab.* 105, 553-558.

13. Abe, Y., Aoki, Y., Kuriyama, S., Kawame, H., Okamoto, N., Kurosawa, K., Ohashi, H., Mizuno, S., Ogata, T., Kure, S., Niihori, T. and Matsubara, Y. and Costello and CFC syndrome study group in Japan. (2012) Prevalence and clinical features of Costello syndrome and cardio-facio-cutaneous syndrome in Japan: findings from a nationwide epidemiological survey. *Am. J. Med. Genet. A.* 158A, 1083-1094.

14. Metoki, H., Ohkubo, T., Obara, T., Akutsu, K., Yamamoto, M., Ishikuro, M., Sakurai, K., Iwama, N., Katagiri, M., Sugawara, J., Hirose, T., Sato, M., Kikuya, M., Yagihashi, K., Matsubara, Y., Yaegashi, N., Mori, S., Suzuki, M., Imai, Y. and the BOSHI Study Group. (2012) Daily serial hemodynamic data during pregnancy and seasonal variation: the BOSHI study. *Clin. Exp. Hypertens.* 34, 290-296.

15. Saito, Y., Aoki, Y., Muramatsu, H., Makishima, H., Maciejewski, J.P., Imaizumi, M., Rikiishi, T., Sasahara, Y., Kure, S., Niihori, T., Tsuchiya, S., Kojima, S. and Matsubara, Y. (2012) Casitas B-cell lymphoma mutation in childhood T-cell acute lymphoblastic leukemia. *Leuk. Res.* 36, 1009-1015.

16. Asano, M., Fujimura, T., Wakusawa, C., Aoki, Y., Matsubara Y. and Aiba, S. (2012) A case of almost unilateral focal dermal hypoplasia resulting from a novel mutation in the *PORCN* gene. *Acta. Derm. Venereol.* 93, 120-121.

17. Patrinos, G.P., Smith, T.D., Howard, H., Al-Mulla, F., Chouchane, L., Hadjisavvas, A., Hamed, S.A., Li, X.T., Marafie, M., Ramesar, R.S., Ramos, F.J., de Ravel, T., El-Ruby, M.O., Shrestha, T.R., Sobrido, M.J., Tadmouri, G., Witsch-Baumgartner, M., Zilfalil, B.A., Auerbach, A.D., Carpenter, K., Cutting, G.R., Dung, V.C., Grody, W., Hasler, J., Jorde L, Kaput, J., Macek, M., Matsubara, Y., Padilla, C., Robinson, H., Rojas-Martinez, A., Taylor, G.R., Vihinen, M., Weber, T., Burn, J., Qi, M., Cotton, R.G. and Rimoin, D.; International Confederation of Countries Advisory Council. (2012) Human Variome Project country nodes: documenting genetic information within a country. *Hum. Mutat.* 33, 1513-1519.

18. Komatsuzaki, S., Sakamoto, O., Fuse, N., Uematsu, M., Matsubara, Y. and Ohura, T. (2012) Clinical reasoning: a young man with progressive subcortical lesions and optic nerve atrophy. *Neurology.* 79, e63-68.

19. Izumi, R., Niihori, T., Aoki, Y., Suzuki, N., Kato, M., Warita, H., Takahashi, T., Tateyama, M., Nagashima, T., Funayama, R., Abe, K., Nakayama, K., Aoki, M. and Matsubara Y. (2012) Exome sequencing identifies a novel *TTN* mutation in a family with hereditary myopathy with early respiratory failure. *J. Hum. Genet. in press.*

20. Saitsu, H., Kato, M. and Matsumoto, N. (2012) Haploinsufficiency of *STXBP1* and Ohtahara syndrome. *Jasper's basic mechanism of the epilepsies, 4th edition.* 824-834.

21. Sakai, H., Suzuki, S., Mizuguchi, T., Imoto, K., Yamashita, Y., Doi, H., Kikuchi, M., Tsurusaki, Y., Saitsu, H., Miyake, N., Masuda, M. and Matsumoto, N. (2012) Rapid detection of gene mutations responsible for non-syndromic aortic aneurysm and dissection using two different methods: resequencing microarray technology and next-generation sequencing. *Hum. Genet.* 131, 591-599.

22. Tsurusaki, Y., Okamoto, N., Ohashi, H., Kosho, T., Imai, Y., Hibi-Ko, Y., Kaname, T., Naritomi, K., Kawame, H., Wakui, K., Fukushima, Y., Homma, T., Kato, M., Hiraki, Y., Yamagata, T., Yano, S., Mizuno, S., Sakazume, S., Ishii, T., Nagai, T., Shiina, M., Ogata, K., Ohta, T., Niikawa, N., Miyatake, S., Okada, I., Mizuguchi, T., Doi, H., Saitsu, H. and Miyake, N. and Matsumoto, N. (2012) Mutations affecting components of the SWI/SNF complex cause Coffin-Siris syndrome. *Nat. Genet.* 44, 376-378

23. Saitsu, H., Kato, M., Koide, A., Goto, T., Fujita, T., Nishiyama, K., Tsurusaki, Y., Doi, H., Miyake, N., Hayasaka, K. and Matsumoto, N. (2012) Whole exome sequencing identifies *KCNQ2* mutations in Ohtahara syndrome. *Ann. Neurol.* **72**, 298-300.

24. Saitsu, H., Kato, M., Osaka, H., Moriyama, N., Horita, H., Nishiyama, K., Yoneda, Y., Kondo, Y., Tsurusaki, Y., Doi, H., Miyake, N., Hayasaka, K. and Matsumoto, N. (2012) *CASK* aberrations in male patients with Ohtahara syndrome and cerebellar hypoplasia. *Epilepsia* **53**, 1441-1449.

25. Miyake, N., Elcioglu, N.H., Iida, A., Isguven, P., Dai, J., Murakami, N., Takamura, K., Cho, T.-J., Kim, O.-H., Hasegawa, T., Nagai, T., Ohashi, H., Nishimura, G., Matsumoto, N. and Ikegawa, S. (2012) *PAPSS2* mutations cause autosomal recessive brachyolmia. *J. Med. Genet.* **49**, 533-538.

26. Tsurusaki, Y., Kosho, T., Hatasaki, K., Narumi, Y., Wakui, K., Fukushima, Y., Doi, H., Saitsu, H., Miyake, N. and Matsumoto, N. (2013) Exome sequencing in a family with an X-linked lethal malformation syndrome: clinical consequences of hemizygous truncating *OFD1* mutations in male patients. *Clin. Genet.* **83**, 135-144.

27. Tsurusaki, Y., Saitoh, S., Tomizawa, K., Sudo, A., Asahina, N., Shiraishi, H., Ito, J., Tanaka, H., Doi, H., Saitsu, H., Miyake, N. and Matsumoto, N. (2012) A *DYNC1H1* mutation causes a dominant spinal muscular atrophy with lower extremity predominance. *Neurogenet.* **13**, 327-332.

28. Tsurusaki, Y., Kobayashi, Y., Hisano, M., Ito, S., Doi, H., Nakashima, M., Saitsu, H., Matsumoto, N. and Miyake, N. (2012) The diagnostic utility of exome sequencing in Joubert syndrome and related disorders. *J. Hum. Genet . in press.*

29. Miyake, N., Mizuno, S., Okamoto, N., Ohashi, H., Shiina, M., Ogata, K., Tsurusaki, Y., Nakashima, M., Saitsu, H., Niikawa, N. and Matsumoto, N. (2012) *KDM6A* point mutations cause Kabuki syndrome. *Hum Mut.* **34**, 108-110.

30. Miyake, N., Yano, S., Sakai, C., Hatakeyama, H., Matsushima, Y., Shiina, M., Watanabe, Y., Bartley, J., Abdenur, J.E., Wang, R.Y., Chang, R., Tsurusaki, Y., Doi, H., Nakashima, M., Saitsu. H., Ogata, K., Goto, Y. and Matsumoto, N. (2013) Mitochondrial complex III deficiency caused by a homozygous *UQCRC2* mutation presenting with neonatal-onset recurrent metabolic decompensation. *Hum. Mut.* **34**, 446-452.

31. Onuki, R., Yamada, R., Yamaguchi, R., Kanehisa, M. and Shibuya, T. (2012) Population model-based inter-diplotype similarity measure for accurate diplotype clustering. *J. Comput.Biol.* **19**, 55-67.

32. Nishijima, T., Komatsu, H., Higasa, K., Takano, M., Tsuchiya, K., Hayashida, T., Oka, S. and Gatanaga, H. (2012) Single nucleotide polymorphisms in *ABCC2* associate with tenofovir-induced kidney tubular dysfunction in Japanese patients with HIV-1 infection: a pharmacogenetic study. *Clin. Infect. Dis.*, **55**, 1558-1567.

33. Higasa, K., Nikaido, M., Saito, T. L., Yoshimura, J., Suzuki, Y., Suzuki, H., Nishihara, H., Aibara, M., Ngatunga, B. P., Kalombo, H. W., Sugano, S., Morishita, S. and Okada, N. (2012) Extremely slow rate of evolution in the HOX cluster revealed by comparison between Tanzanian and Indonesian coelacanths. *Gene.* **505**, 324-332.

34. Myouzen, K., Kochi, Y., Okada, Y., Terao, C., Suzuki, A., Ikari, K., Tsunoda, T., Takahashi, A., Kubo, M., Taniguchi, A., Matsuda, F., Ohmura, K., Momohara, S., Mimori, T., Yamanaka, H., Kamatani, N., Yamada, R., Nakamura, Y. and Yamamoto, K. (2012) Functional variants in *NFKBIE* and *RTKN2* involved in activation of the NF-κB pathway are associated with rheumatoid arthritis in Japanese. *PLoS Genet.* **8**, e1002949.

35. Terao, C., Hashimoto, M., Yamamoto, K., Murakami, K., Ohmura, K., Nakashima, R., Yamakawa, N., Yoshifuji, H., Yukawa, N., Kawabata, D., Usui, T., Yoshitomi, H., Furu, M., Yamada, R., Matsuda, F., Ito, H., Fujii, T. and Mimori, T. (2013) Three groups in the 28 joints for rheumatoid arthritis synovitis - analysis using more than 17,000 assessments in the KURAMA database. *PLoS One.* **8**, e59341.

36. Cui, J., Stahl, E.A., Saevarsdottir, S., Miceli, C., Diogo, D., Trynka, G., Raj, T., Mirkov, M.U., Canhao, H., Ikari, K., Terao, C., Okada, Y., Wedrén, S., Askling, J., Yamanaka, H., Momohara, S., Taniguchi, A., Ohmura, K., Matsuda, F., Mimori, T., Gupta, N., Kuchroo, M., Morgan, A.W., Isaacs, J.D., Wilson,

A.G., Hyrich, K.L., Herenius, M., Doorenspleet, M.E., Tak, P.P., Crusius, J.B., van der Horst-Bruinsma, I.E., Wolbink, G.J., van Riel, P.L., van de Laar, M., Guchelaar, H.J., Shadick, N.A., Allaart, C.F., Huizinga, T.W., Toes, R.E., Kimberly, R.P., Bridges, S.L. Jr, Criswell, L.A., Moreland, L.W., Fonseca, J.E., de Vries, N., Stranger, B.E., De Jager, P.L., Raychaudhuri, S., Weinblatt, M.E., Gregersen, P.K., Mariette, X., Barton, A., Padyukov, L., Coenen, M.J., Karlson, E.W. and Plenge R.M. (2013) Genome-wide association study and gene expression analysis identifies *CD84* as a predictor of response to etanercept therapy in rheumatoid arthritis. *PLoS Genet.* 9, e1003394.

PLD4 as a Novel Susceptibility Gene for Systemic Sclerosis in a Japanese Population

Chikashi Terao,[1] Koichiro Ohmura,[1] Yasushi Kawaguchi,[2] Tetsuya Nishimoto,[3] Aya Kawasaki,[4] Kazuhiko Takehara,[5] Hiroshi Furukawa,[6] Yuta Kochi,[7] Yuko Ota,[2] Katsunori Ikari,[2] Shinichi Sato,[8] Shigeto Tohma,[6] Ryo Yamada,[1] Kazuhiko Yamamoto,[8] Michiaki Kubo,[7] Hisashi Yamanaka,[2] Masataka Kuwana,[3] Naoyuki Tsuchiya,[4] Fumihiko Matsuda,[1] and Tsuneyo Mimori[1]

Objective. Systemic sclerosis (SSc) is an autoimmune disease for which multiple susceptibility genes have been reported. Genome-wide association studies have shown that large numbers of susceptibility genes are shared among autoimmune diseases. Recently, our group identified 9 novel susceptibility genes associated with rheumatoid arthritis (RA) in a Japanese population. The aim of this study was to elucidate whether the 18 genes that displayed associations or suggestive associations for RA in our previous study are associated with SSc in Japanese.

Methods. We performed an association study that included 415 patients with SSc and 16,891 control subjects, followed by a replication study that included

315 patients and 21,054 control subjects. The 18 markers reported to display association with RA were analyzed for their associations with SSc in the first study, and 5 markers were further analyzed in the replication study. The inverse variance method was used to evaluate the associations of these markers with SSc in a combined study.

Results. In the phospholipase D4 gene (*PLD4*), rs2841277 displayed a significant association with SSc in Japanese patients ($P = 0.00017$). We observed that rs2841280 in exon 2 of *PLD4* was in strong linkage disequilibrium with rs2841277 and introduced an amino acid alteration. We also observed associations between SSc and rs6932056 in *TNFAIP3* and rs2280381 in *IRF8* ($P = 0.0000095$ and $P = 0.0030$, respectively), both of which displayed associations with SSc in a European population.

Conclusion. We determined that *PLD4* is a novel susceptibility gene for SSc in Japanese, thus confirming the involvement of *PLD4* in autoimmunity. Associations between SSc and *TNFAIP3* or *IRF8* were also detected in our Japanese population. SSc and RA appear to share relatively large proportions of their genetic backgrounds.

Systemic sclerosis (SSc) is a connective tissue disease that affects 7–489 individuals per million worldwide and is characterized by the excess production of extracellular matrix molecules and fibrosis (1). Patients with SSc display skin sclerosis, obliterative microvasculopathy such as Raynaud's phenomenon, and multiorgan involvement. Severe complications of SSc sometimes develop, including interstitial lung disease, pulmonary hypertension, and renal crisis. These severe symptoms

and complications of SSc result in a poor prognosis and a shortened lifespan (2,3). No effective method for preventing or curing SSc has been established (4).

It is well known that SSc has genetic components (5); for example, a US study revealed that the incidence of SSc was much higher among the families of patients with SSc compared with the general population (6). Recent technologic developments enabled the use of genome-wide association studies (GWAS) to identify novel susceptibility loci for autoimmune diseases (7). GWAS of European patients with SSc revealed that *CD247* (8), HLA (8), *TNIP1*, *PSORS1C1*, and *RHOB* (9) are susceptibility loci for SSc. In addition, another GWAS identified associations between *IRF8*, *GRB10*, and *SOX5* and limited cutaneous SSc (lcSSc) in a European population (10). Furthermore, studies adopting a candidate gene approach based on subjecting genes to functional inference analysis led to the identification of *STAT4* (11), *IRF5* (12), *TBX21* (13), *NLRP1* (14), *TNFSF4* (15), *CD226* (16), *BLK* (17), and *TNFAIP3* (18) as novel susceptibility genes for SSc in Europeans. SSc association studies in Japanese populations confirmed that *STAT4* (19), *IRF5* (20), and *BLK* (21) are associated with SSc and identified *UBE2L3* as a susceptibility gene for diffuse cutaneous SSc (dcSSc) (22). An association between HLA and SSc was also detected in Asians (23). These findings suggest a clear overlap in the genetic background of SSc between different populations.

It is well known that susceptibility genes are shared by various autoimmune diseases (24). In fact, HLA (25), *STAT4* (26), and *TNFAIP3* (27,28), which are susceptibility genes for SSc, have also been reported to be associated with rheumatoid arthritis (RA). In addition, *PTPN22*, which was shown to be strongly associated with RA in a European population (29), showed a suggestive association with SSc in Europeans (30). The sharing of these susceptibility genes between RA and SSc raises the possibility that newly identified susceptibility genes for RA could also be susceptibility genes for SSc. Recently, a large Japanese consortium, the Genetic and Allied research in Rheumatic diseases Networking consortium, identified 9 novel susceptibility genes and 6 candidate susceptibility genes for RA using a meta-analysis of GWAS and replication studies (31). Four other genes, namely, HLA, *PADI4*, *CCR6*, and *TNFAIP3*, were also confirmed to display associations with RA. Here, we performed a 2-stage association study of Japanese patients with SSc, in which we genotyped these genes as candidate susceptibility loci.

PATIENTS AND METHODS

Study subjects. DNA samples were obtained from 415 patients with SSc at Kyoto University Hospital and Tokyo Women's Medical University; these samples comprised the first set. Independent DNA samples were obtained from 315 patients with SSc at Keio University Hospital, Sagamihara National Hospital, and Kanazawa University Hospital; these samples were used as the replication set. All patients were Japanese, all had a diagnosis of SSc as determined by a rheumatologist, and all fulfilled the 1980 American College of Rheumatology classification criteria for SSc (32). The patients with SSc for whom clinical information was available were classified as having lcSSc or dcSSc, according to the definitions developed by LeRoy et al (33). The control samples were described in detail in our previous study (31). The current study was approved by the local ethics committees at each institution, and written informed consent was obtained from all subjects. The basic characteristics of the study subjects are shown in Table 1.

Genotyping. The 9 novel susceptibility markers, 6 potentially associated markers, and 4 confirmed markers of RA that were identified in our previous study in a Japanese population (31) were chosen as candidate susceptibility markers for SSc in Japanese. Eighteen of the 19 markers (HLA was excluded; see Results), none of which had previously been reported to be associated with SSc in Japanese individuals, were genotyped in the current study. The 5 candidate markers in the first set that showed associations with *P* values less than 0.1 were further genotyped in the replication study. Single-nucleotide polymorphisms (SNPs) rs2841280 and rs894037 were chosen as candidate causative variants in the phospholipase D4 gene (*PLD4*) region. Because rs894037 was shown to be monomorphic in Japanese, rs2841280 was genotyped in 334 control subjects, in addition to all patients, for imputation reference. The patients in the first and replication studies were genotyped at Kyoto University or Tokyo Women's Medical University and at Keio University or University of Tsukuba, respectively, using TaqMan assays (Applied Biosystems). The genotyping methods in control subjects were described in detail in our previous study (31).

Briefly, control genotypes in the first set were imputed based on the genome-scanning data, using mach2dat software with HapMap Phase II East Asian Populations as reference. The control genotypes for the replication study were extracted from genome-scanning data for the markers included on Illumina HumanHap610 Quad BeadChips. The genotypes for rs6932056 (which is not included in the array) were imputed based on the genome-scanning data, using mach2dat software with HapMap Phase II East Asian Populations as reference, and were used as control data for the replication set. The genotypes for rs2841280 (which is not included in the HapMap data or the array) were also imputed in control subjects, based on the genome-scanning data, using mach2dat software. Genotyping data for the 334 control subjects as determined by TaqMan assay in combination with genome-scanning data were used as reference.

Statistical analysis. The associations between the genotyped markers and SSc were analyzed using a Cochran-Armitage trend test in both the first and replication studies. Subanalyses were performed by comparing the genotypes of

Table 1. Characteristics of the study population*

	Patients	Controls
First set		
Institutions	Kyoto University, Tokyo Women's Medical University	Kyoto University, Tokyo Women's Medical University, BioBank Japan
Typing	TaqMan assay	Illumina HumanHap610 Quad BeadChip, Illumina HumanHap550 BeadChip, Affymetrix Genome-Wide Human SNP Array 6.0
Limited SSc/diffuse SSc, %	49.6/50.4	Not applicable
Anti–topo I/ACA, %	30.6/31.1	Not applicable
Interstitial lung disease, %	48.9	Not applicable
Age, mean ± SD years	50.9 ± 14.7	60.9 ± 12.5
Female, %	91.3	44.9
Replication set		
Institutions	Keio University, Sagamihara National Hospital, Kanazawa University	Kyoto University, BioBank Japan
Typing	TaqMan assay	Illumina HumanHap550 BeadChip, Illumina HumanHap610 Quad BeadChip
Limited SSc/diffuse SSc, %	63.8/34.6	Not applicable
Anti–topo I/ACA, %	29.5/35.2	Not applicable
Interstitial lung disease, %	43.2	Not applicable
Age, mean ± SD years	51.4 ± 14.1	59.3 ± 14.2
Female, %	87.3	48.4

* The first set included 415 patients with systemic sclerosis (SSc) and 16,891 control subjects. The replication set included 315 patients with SSc and 21,054 control subjects. Anti–topo I = anti–topoisomerase I; ACA = anticentromere antibody.

the control subjects with those of patients in the SSc subgroups based on the disease phenotypes. The subanalyses used the same control subjects as were used in the association studies. Intracase analyses based on phenotypes were also performed.

Odds ratios (ORs) and 95% confidence intervals were also calculated. The associations detected in the first and replication studies were then meta-analyzed using the inverse variance method. The resultant P values were corrected using the Benjamini-Hochberg false discovery rate (FDR) criterion, and corrected P values less than 0.05 were regarded as significant in both the combined study and the subanalyses. The efficiency of the current study was estimated by calculating the likelihood of detecting 3 significant markers (after correcting the P values using the FDR method) among 18 randomly selected markers. After the statistically significant markers were identified, the best-fit model for each association was analyzed using dominant, recessive, trend, and allelic chi-square tests or models. Statistical analyses were performed using R or SPSS (version 18) software.

RESULTS

Analyses of candidate genes for SSc in a Japanese population. The 415 patients with SSc and 16,891 control subjects in the first set were genotyped for the 18 markers that were shown to have associations or suspected associations with RA in our previous study. The HLA region was excluded from the genotyped markers, because this region has already been shown to be associated with SSc in Asians. The allele frequencies of

the patients were compared with those of the control subjects, using a Cochran-Armitage trend test.

As a result, 3 markers that demonstrated associations with P values less than 0.01 in the first set (Table 2) were identified, namely, rs6932056 in the *TNFAIP3* region ($P = 0.0000038$, OR 1.69), rs10821944 in the *ARID5B* region ($P = 0.0025$, OR 1.25), and rs2841277 in the *PLD4* region ($P = 0.0054$, OR 1.25). Two loci that showed suggestive associations with P values less than 0.1 (Table 2) were also identified, namely, rs12529514 in the *CD83* region ($P = 0.083$, OR 1.18) and rs2280381 in the *IRF8* region ($P = 0.095$, OR 1.19). The *TNFAIP3* and *IRF8* regions were previously reported to display associations with SSc and lcSSc, respectively, in European populations (10,18). These 5 markers were selected as candidate susceptibility markers for SSc in Japanese and were subjected to validation.

Next, a replication study consisting of 315 patients with SSc and 21,054 control subjects was performed to validate the associations of the 5 markers with SSc. The patients were genotyped for the 5 markers. The genotypes of the control subjects for the 5 markers, except rs6932056, were extracted from the Illumina Infinium HumanHap610 Quad array, as reported previously (31). The genotypes for rs6932056 were imputed based on genome-scanning data using mach2dat soft-

Table 2. Association studies of Japanese patients with SSc*

SNP	Chr	Gene	Allele 1/2	First set			Replication set			Combined study		
				Controls	Patients	*P*	Controls†	Patients	*P*	*P*, patients vs. controls	OR (95% CI)	*P*, patients without overlapping RA vs. controls
rs766449	1	*PADI4*	T/C	0.40	0.37	0.12	–	–	–	–	–	–
rs11900673	2	*B3GNT2*	T/C	0.29	0.28	0.65	–	–	–	–	–	–
rs2867461	4	*ANXA3*	A/G	0.44	0.43	0.57	–	–	–	–	–	–
rs657075	5	*IL3-CSF2*	A/G	0.36	0.34	0.25	–	–	–	–	–	–
rs12529514	6	*CD83*	C/T	0.14	0.16	0.083	0.15	0.16	0.31	0.046	1.15 (1.00–1.33)	0.040
rs1571878	6	*CCR6*	C/T	0.49	0.47	0.28	–	–	–	–	–	–
rs6932056	6	*TNFAIP3*	C/T	0.069	0.11	3.8×10^{-6}	0.067	0.079	0.23	9.5×10^{-6}	1.50 (1.25–1.80)	5.4×10^{-6}
rs2233434	6	*NFKBIE*	G/A	0.21	0.21	0.93	–	–	–	–	–	–
rs10821944	10	*ARID5B*	G/T	0.36	0.41	0.0025	0.36	0.37	0.64	0.0073	1.16 (1.04–1.29)	0.010
rs3781913	11	*PDE2A-CENTD2*	T/G	0.69	0.69	0.91	–	–	–	–	–	–
rs4937362	11	*ETS1-FLI1*	T/C	0.68	0.68	0.88	–	–	–	–	–	–
rs2841277	14	*PLD4*	T/C	0.69	0.74	0.0054	0.69	0.73	0.012	0.00017	1.25 (1.11–1.41)	0.00052
rs3783637	14	*GCH1*	C/T	0.74	0.73	0.54	–	–	–	–	–	–
rs1957895	14	*PRKCH*	G/T	0.39	0.41	0.26	–	–	–	–	–	–
rs6496667	15	*ZNF774*	A/C	0.35	0.37	0.33	–	–	–	–	–	–
rs7404928	16	*PRKCB1*	T/C	0.62	0.63	0.51	–	–	–	–	–	–
rs2280381	16	*IRF8*	T/C	0.84	0.86	0.095	0.83	0.87	0.0099	0.0030	1.26 (1.08–1.47)	0.0021
rs2847297	18	*PTPN2*	G/A	0.34	0.34	0.85	–	–	–	–	–	–

* SSc = systemic sclerosis; SNP = single-nucleotide polymorphism; Chr = chromosome; OR = odds ratio; 95% CI = 95% confidence interval; RA = rheumatoid arthritis.
† The control rs6932056 genotypes used in the replication study were imputed using genome-scanning data obtained for 3,765 subjects.

ware, because rs6932056 was not included in the array. As a result, rs2841277 in the *PLD4* region and rs2280381 in the *IRF8* region showed relatively strong associations with SSc (*P* = 0.012, OR 1.25 and *P* = 0.0099, OR 1.37, respectively) (Table 2). Interestingly, we observed that all 5 of the markers that displayed associations in the first study also demonstrated the same association directions in the replication study.

The inverse variance method was used to combine the data for the first and replication studies. SNPs rs2841277 in the *PLD4* region, rs6932056 in the *TNFAIP3* region, and rs2280381 in the *IRF8* region showed significant associations with SSc even after correcting the associated *P* values using the FDR method for multiple testing (Table 2). Importantly, all 3 of these loci shared risk alleles with RA. Although rs6932056 in the *TNFAIP3* region did not show a strong association with SSc in the replication study, its association was significant in the combined study. The *PLD4* region was shown to be a novel susceptibility gene for SSc, and, for the first time, the *TNFAIP3* and *IRF8* regions were confirmed to be associated with SSc in Japanese.

The association between rs2841277 and SSc was then investigated in detail. When the 200-kbp region around rs2841277 was evaluated, 2 hypothetical genes

and cell division cycle associated 4 gene (*CDCA4*) were located at the region, in addition to *PLD4*. *PLD4* was the only gene whose region showed moderate to strong linkage disequilibrium (LD) with rs2841277, indicating *PLD4* as a susceptibility gene (Figure 1A). We vigorously searched candidate markers in exons of *PLD4* that showed strong LD with rs2841277 and selected 2 markers registered in the 1000 Genomes Project (34) that displayed >5% frequency in genotyped subjects, namely, rs2841280 (Figure 1B) and rs894037 in exon 2. Genotyping of these polymorphisms revealed strong LD between rs2841280 (E27Q) and rs2841277 (D' = 0.98, $r^2 = 0.75$) and monomorphism of rs894037 in Japanese. An association study of rs2841280 using control genotypes obtained by imputation supported association of *PLD4* with SSc (*P* = 6.3 × 10^{-5}) (see Supplementary Tables 1 and 2, available on the *Arthritis & Rheumatism* web site at http://onlinelibrary.wiley.com/doi/10.002/art.37777/abstract).

Because the 3 loci were associated with RA in a Japanese population, we analyzed whether the associations with SSc in the current study were contributed by patients with both RA and SSc. When 22 patients who had RA as well as SSc were excluded, significant associations for the 3 loci were still observed (Table 2). A
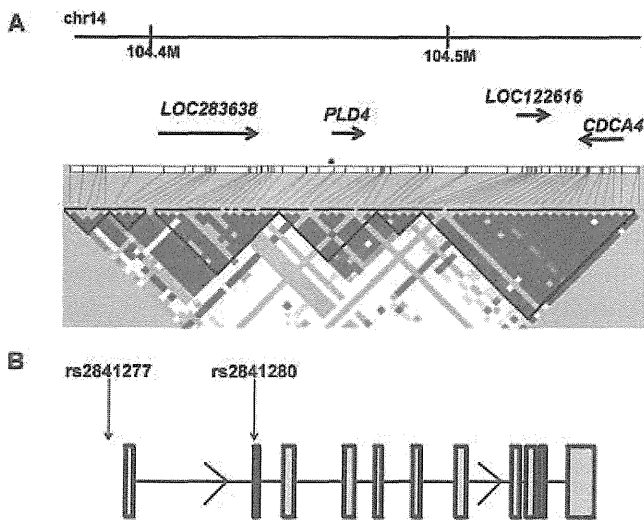
Figure 1. Linkage disequilibrium (LD) block around the *PLD4* region and the *PLD4* structure. A, LD block and genes around *PLD4*. The LD block is based on HapMap phase 3 data. **Asterisk** indicates rs2841277. B, Schematic view of *PLD4* structure. Rectangles represent exons of *PLD4*.

further stringent analysis excluding patients with other autoimmune diseases demonstrated significant associations of the 3 genes (see Supplementary Table 2). When we compared SSc patients with and those without other autoimmune diseases for the associated alleles, no differences were observed (data not shown).

Subanalysis of types of SSc. Previous studies have revealed that the genetic background of SSc varies between different types of SSc (11,18). Thus, subanalyses of the 5 regions examined in the combined study were performed, in which the allele frequencies of the control subjects were compared with those of the patients with lcSSc or dcSSc. The control subjects were the same as those used in the first study or the combined study. Although *PLD4* and *TNFAIP3* did not display a preference for either SSc phenotype, *IRF8* and *ARID5* showed suggestive preferences for lcSSc, and *CD83* showed a suggestive preference for dcSSc (Table 3).

We also investigated whether the susceptibility loci affect autoantibody status and severe complications. The association studies revealed an association of *TNFAIP3* with SSc patients who possess anticentromere antibodies (ACAs) (see Supplementary Table 3, available on the *Arthritis & Rheumatism* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.37777/abstract), but intracase analyses did not demonstrate clear significance ($P = 0.043$). We did not observe other associations between the susceptibility loci and clinical phenotypes of SSc, in either case–control analyses or intracase analyses.

Efficacy of the current study. In the current study, a candidate gene analysis was performed based on a meta-analysis of RA GWAS, because many susceptibility genes for autoimmune disease have been reported

Table 3. Associations of the 2 SSc subtypes*

SNP	Chr	Gene	Allele 1/2	Controls, allele 1 frequency	Limited cutaneous SSc (n = 408)			Diffuse cutaneous SSc (n = 318)		
					Allele 1 frequency	P	OR (95% CI)	Allele 1 frequency	P	OR (95% CI)
rs766449	1	PADI4	T/C	0.40	0.39	0.52	0.94 (0.77–1.14)	0.36	0.11	0.85 (0.69–1.04)
rs11900673	2	B3GNT2	T/C	0.29	0.25	0.096	0.82 (0.66–1.03)	0.31	0.32	1.11 (0.9–1.38)
rs2867461	4	ANXA3	A/G	0.44	0.42	0.40	0.92 (0.75–1.12)	0.44	0.97	1.00 (0.82–1.22)
rs657075	5	IL3-CSF2	A/G	0.36	0.34	0.54	0.94 (0.76–1.15)	0.33	0.23	0.88 (0.72–1.08)
rs12529514	6	CD83	C/T	0.14	0.15	0.79	1.03 (0.85–1.25)	0.18	0.0075	1.32 (1.08–1.62)
rs1571878	6	CCR6	C/T	0.49	0.48	0.81	0.98 (0.80–1.19)	0.46	0.20	0.88 (0.72–1.07)
rs6932056	6	TNFAIP3	C/T	0.069	0.093	0.0062	1.40 (1.1–1.78)	0.10	0.00063	1.57 (1.21–2.04)
rs2233434	6	NFKBIE	G/A	0.21	0.20	0.60	0.94 (0.73–1.20)	0.22	0.70	1.05 (0.83–1.33)
rs10821944	10	ARID5B	G/T	0.36	0.40	0.0085	1.22 (1.05–1.41)	0.38	0.30	1.09 (0.93–1.29)
rs3781913	11	PDE2A-CENTD2	T/G	0.69	0.69	0.98	1.00 (0.81–1.24)	0.69	0.90	1.01 (0.82–1.25)
rs2841277	14	PLD4	T/C	0.69	0.73	0.0067	1.24 (1.06–1.45)	0.74	0.0049	1.29 (1.08–1.55)
rs2841280	14	PLD4	C/G	0.64	0.69	0.0011	1.30 (1.11–1.52)	0.69	0.0086	1.27 (1.06–1.51)
rs2847297	18	PTPN2	G/A	0.34	0.33	0.67	0.96 (0.78–1.18)	0.34	0.87	1.02 (0.83–1.25)
rs4937362	11	ETS1-FLI1	T/C	0.68	0.68	0.75	0.97 (0.78–1.19)	0.69	0.92	1.01 (0.82–1.25)
rs3783637	14	GCH1	C/T	0.74	0.73	0.69	0.96 (0.77–1.19)	0.73	0.65	0.95 (0.76–1.18)
rs1957895	14	PRKCH	G/T	0.39	0.40	0.84	1.02 (0.84–1.25)	0.42	0.16	1.15 (0.95–1.41)
rs6496667	15	ZNF774	A/C	0.35	0.39	0.088	1.19 (0.97–1.45)	0.34	0.75	0.97 (0.79–1.19)
rs7404928	16	PRKCB1	T/C	0.62	0.61	0.60	0.95 (0.78–1.16)	0.66	0.15	1.17 (0.95–1.44)
rs2280381	16	IRF8	T/C	0.84	0.88	0.0038	1.36 (1.11–1.68)	0.86	0.21	1.16 (0.92–1.45)

* SSc = systemic sclerosis; SNP = single-nucleotide polymorphism; Chr = chromosome; OR = odds ratio; 95% CI = 95% confidence interval.
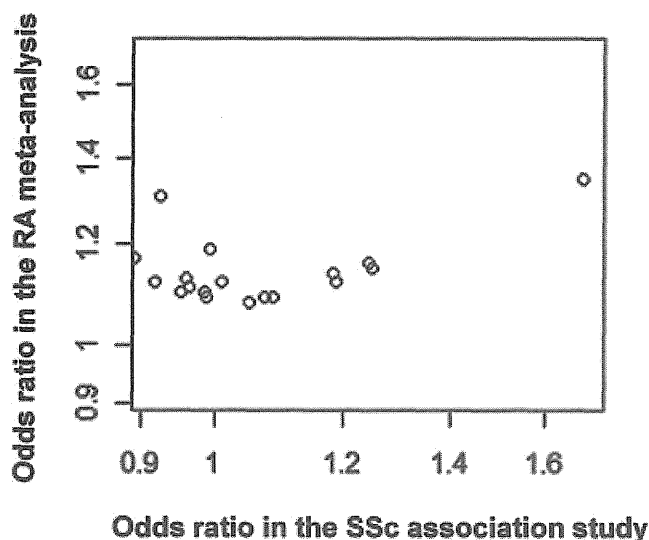
Figure 2. Comparison of associations for systemic sclerosis (SSc) and rheumatoid arthritis (RA). The odds ratios obtained for 18 genes in association studies of SSc and RA are plotted.

to be shared by a wide range of diseases. As a result, 3 susceptibility genes for SSc in Japanese were identified. Thus, we analyzed whether the candidate gene approach taken in the current study for detecting novel susceptibility genes for SSc was effective. When the likelihood of finding 3 susceptibility genes among 18 genes by chance was calculated, the likelihood was determined to be 2.5×10^{-8}. These results indicated that our approach to identifying novel susceptibility genes for systemic diseases is effective. It would be interesting to compare the risk direction of the genotyped markers between RA and SSc. Although the 3 susceptibility loci for SSc shared risk direction with RA, no correspondence of the risk directions of the markers between the 2 diseases was detected (Figure 2). This indicated that a large proportion of the 18 RA markers are not shared by SSc, and that the lack of association between the 13 markers and SSc was not attributable to the low power produced by the relatively small number of SSc patients included in this study.

DISCUSSION

Because SSc can lead to severe complications, poor quality of life, and shortened survival, clarifying the characteristics of SSc is important. Clarification of the disease would aid the search for novel therapeutic targets and the development of new therapeutic strategies. Detecting susceptibility genes using GWAS or a candidate gene approach would also help to uncover the pathophysiology underlying SSc.

Previous studies have revealed that more than 15 markers and loci are associated with SSc. However, the markers detected so far cannot fully explain the genetics of SSc, indicating that many susceptibility genes are yet to be identified. Because a relatively large proportion of RA susceptibility genes are shared by other autoimmune diseases (24), a candidate gene approach using novel markers observed in GWAS of RA is a fascinating way of identifying new SSc markers. In fact, some of the novel susceptibility markers for RA identified in the meta-analysis were shown to be susceptibility markers for systemic lupus erythematosus (SLE) and Graves' disease (31).

In the current study, we successfully identified 3 susceptibility genes for SSc in Japanese. No studies have identified *PLD4* as an SSc-associated locus. The current study is also the first to detect *TNFAIP3* and *IRF8* as susceptibility genes for SSc in a Japanese population. The best-fit models for each association are shown in Supplementary Table 4, available on the *Arthritis & Rheumatism* web site at http://onlinelibrary.wiley.com/doi/10.002/art.37777/abstract.

It is conceivable that these 3 associations might have been obtained due to the overlap of RA and SSc. Even after excluding the patients with both RA and SSc based on physicians' reports, the significant associations for the 3 loci were still observed (Table 3). Information regarding rheumatoid factor (RF) and anti–citrullinated protein antibody (ACPA) was available for 371 SSc patients without RA and 65 SSc patients without RA, respectively, of whom 21.6% and 10.8% were positive for RF and ACPA, respectively. These prevalences are compatible with those previously observed in SSc patients without RA (35,36). Moreover, we showed that the effect sizes and risk direction of the markers tested in this study were dissociated between SSc and RA. In addition, further stringent analysis comprising SSc patients without any autoimmune disease also showed the associations of the 3 loci. These results indicate that the associations of the 3 loci are not attributable to overlapping of RA or other diseases.

Although the associations of the *ARID5B* and *CD83* loci with SSc did not reach a stringently significant level in the combined study, the tendencies toward an association with SSc displayed by rs10821944 in the *ARID5B* locus and rs12529514 in the *CD83* region in the first study were maintained in the replication study. This indicates that these loci are potential susceptibility regions for SSc. Further replication studies are needed to

address the associations of these 2 loci with SSc in a Japanese population.

Because *TNFAIP3* was reported to be strongly associated with SSc in a European population (18), the significant associations detected in the combined study indicate that *TNFAIP3* displays general associations with SSc that go beyond ethnic boundaries. In addition, rs6932056, which displayed a strong association with SSc in a European population (18), is in strong LD with rs5029939 ($r^2 = 0.85$) in the Japanese population. SNP rs6932056 also displays strong LD with rs2230926, a missense mutation of *TNFAIP3* ($r^2 = 0.85$), in Japanese. The rs2230926 missense mutation leads to an amino acid alteration in the OTU (ovarian tumor) domain of the A20 protein, which is considered to result in decreased NF-κB signaling. Because we did not observe strong associations between rs6932056 and SSc in the replication study, it will be necessary to reexamine the association between *TNFAIP3* and SSc using independent sample sets of Japanese patients with SSc, in spite of the significant associations detected in this study.

PLD4 is a recently reported member of the phospholipase family without phospholipase D activity. *PLD4* is expressed in the spleen and early postnatal microglia in the white matter of mice (37). The phenotypes of *Pld4*-deficient mice have not been reported. In addition, little is known about the expression or distribution of *PLD4* in humans. Although the functions of *PLD4* are also poorly understood, it is known to be involved in the phagocytosis of microglia (38). The expression of *PLD4* around the marginal zone in the spleen might support the functional involvement of *PLD4* in immunologic systems. It is interesting that rs2841280, which alters an amino acid of PLD-4, is associated with SSc. Minor allele G of rs2841280 is associated in a protective manner. The impact of an amino acid alteration brought by rs2841280 on the effect of PLD-4 protein is not known.

When we analyzed the impact of the amino acid alteration using in silico analysis (SIFT software; http://sift.jcvi.org/), it was shown to result in a small effect. However, the association raises the possibility that this polymorphism leads functional modulation of PLD-4, and it is feasible to analyze the functional change of PLD-4 protein with rs2841280, using animal models of SSc. When we performed an in silico analysis of the effect of rs2841277 and rs2841280 on *PLD4* expression, we did not detect any clear associations between the 2 genotypes and *PLD4* transcription ($P > 0.05$) (39). Therefore, in spite of the association of these 2 muta-

tions, it has not been confirmed whether one of these 2 polymorphisms is the causative mutation.

Although the detection of a *P* value less than 5×10^{-8} in a GWAS is stringent evidence of an association between a marker and a particular disease, the detection of suggestive associations between the *PLD4* region and SSc in European GWAS would indicate that associations exist between *PLD4* and SSc in other populations. However, when we examined the associations between the *PLD4* locus or nearby loci and SSc in GWAS involving a European population, we did not detect any strong associations ($P < 10^{-4}$) (8,9). According to the HapMap database, the European population displays a higher risk allele frequency for rs2841277 than the Japanese population. In addition, the HapMap database also indicates that the LD block spanning *PLD4*, which includes rs2841277, is similar in Europeans and Japanese. Nevertheless, a European population did not show a strong association between *PLD4* and SSc, suggesting that *PLD4* has a stronger effect on autoimmune diseases in Japanese than in Europeans. There is also a possibility that these 2 polymorphisms are only markers, and that a rare variant in LD with the 2 markers affects disease onset. A rare causative variant might explain a different association of *PLD4* with SSc between populations.

IRF8 was shown to be associated with SLE in a European population (40). Interferon regulatory factor 8 (IRF-8) protein is a transcription factor involved in the interferon pathway. The interferon pathway has been shown to be involved with a broad range of autoimmune diseases, including SSc (41). Thus, it is interesting that *IRF5* and *IRF8*, both of which belong to the IRF family, displayed associations with SSc. Although a European GWAS of SSc patients revealed suggestive associations between the *IRF4* locus and SSc, the results were not successfully replicated (8), indicating that the different functional roles of each IRF family molecule might influence the development of SSc. *IRF8* promotes B cell differentiation; however, the roles and importance of B cells in skin fibrosis in SSc patients have not been established (42–44). *IRF8* and its mutant variants are also known to be involved in the development of dendritic cells (45). Thus, the association between *IRF8* and SSc might indicate the involvement of B cells and dendritic cells in the development of SSc.

When the patients with SSc were classified as having either lcSSc or dcSSc and subanalyses were performed, *ARID5B*, *IRF8*, and *CD83* displayed stronger associations with one of the 2 phenotypes. However, the associations of these 3 markers with the phenotypes

were not strong enough to provide convincing evidence of a clear distinction between the genetic backgrounds of the 2 SSc phenotypes. When the associations of the SSc subtypes with the other 13 markers in the first set were analyzed, no strong association was detected ($P > 0.05$). Other subanalyses of the susceptibility loci in the combined set did not show significant results between disease phenotypes, due to lack of power. Because classification according to disease phenotypes resulted in limited numbers of subjects in each subset, we conducted this subanalysis only in the combined set. The association between *TNFAIP3* and ACAs should be confirmed in a large-scale association study.

Although GWAS are an extremely powerful way to detect novel susceptibility genes for diseases, GWAS of patients with SSc have been performed only in European populations. Our study detected strong evidence for the sharing of susceptibility genes between RA and SSc in a Japanese population. In addition, the current study indicated that a candidate gene approach based on the results of GWAS of other diseases that display pathologic signaling pathways or mechanisms similar to those associated with the disease being examined is an effective approach to identifying novel susceptibility genes.

It will be interesting to perform GWAS of Japanese patients with SSc and analyze the similarities and differences in the detected associations not only between Japanese and Europeans but also between Japanese patients with SSc and Japanese patients with RA.

ACKNOWLEDGMENT

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.
Study conception and design. Terao, Ohmura, Kawaguchi, Nishimoto, Kawasaki, Takehara, Furukawa, Kochi, Ota, Ikari, Sato, Tohma, Yamada, Yamamoto, Kubo, Yamanaka, Kuwana, Tsuchiya, Matsuda, Mimori.
Acquisition of data. Terao, Ohmura, Kawaguchi, Nishimoto, Kawasaki, Takehara, Furukawa, Kochi, Ota, Ikari, Sato, Tohma, Yamada, Yamamoto, Kubo, Yamanaka, Kuwana, Tsuchiya, Matsuda, Mimori.
Analysis and interpretation of data. Terao, Ohmura.

REFERENCES

1. Chifflot H, Fautrel B, Sordet C, Chatelus E, Sibilia J. Incidence and prevalence of systemic sclerosis: a systematic literature review. Semin Arthritis Rheum 2008;37:223–35.

2. Kawut SM, Taichman DB, Archer-Chicko CL, Palevsky HI, Kimmel SE. Hemodynamics and survival in patients with pulmonary arterial hypertension related to systemic sclerosis. Chest 2003;123: 344–50.

3. Ioannidis JP, Vlachoyiannopoulos PG, Haidich AB, Medsger TA Jr, Lucas M, Michet CJ, et al. Mortality in systemic sclerosis: an international meta-analysis of individual patient data. Am J Med 2005;118:2–10.

4. Bhattacharyya S, Wei J, Varga J. Understanding fibrosis in systemic sclerosis: shifting paradigms, emerging opportunities. Nat Rev Rheumatol 2012;8:42–54.

5. Romano E, Manetti M, Guiducci S, Ceccarelli C, Allanore Y, Matucci-Cerinic M. The genetics of systemic sclerosis: an update. Clin Exp Rheumatol 2011;29:S75–86.

6. Arnett FC, Cho M, Chatterjee S, Aguilar MB, Reveille JD, Mayes MD. Familial occurrence frequencies and relative risks for systemic sclerosis (scleroderma) in three United States cohorts. Arthritis Rheum 2001;44:1359–62.

7. Terao C, Ohmura K, Katayama M, Takahashi M, Kokubo M, Diop G, et al. Myelin basic protein as a novel genetic risk factor in rheumatoid arthritis: a genome-wide study combined with immunological analyses. PLoS One 2011;6:e20457.

8. Radstake TR, Gorlova O, Rueda B, Martin JE, Alizadeh BZ, Palomino-Morales R, et al. Genome-wide association study of systemic sclerosis identifies CD247 as a new susceptibility locus. Nat Genet 2010;42:426–9.

9. Allanore Y, Saad M, Dieude P, Avouac J, Distler JH, Amouyel P, et al. Genome-wide scan identifies TNIP1, PSORS1C1, and RHOB as novel risk loci for systemic sclerosis. PLoS Genet 2011;7:e1002091.

10. Gorlova O, Martin JE, Rueda B, Koeleman BP, Ying J, Teruel M, et al. Identification of novel genetic markers associated with clinical phenotypes of systemic sclerosis through a genome-wide association strategy. PLoS Genet 2011;7:e1002178.

11. Rueda B, Broen J, Simeon C, Hesselstrand R, Diaz B, Suarez H, et al. The STAT4 gene influences the genetic predisposition to systemic sclerosis phenotype. Hum Mol Genet 2009;18:2071–7.

12. Dieude P, Guedj M, Wipff J, Avouac J, Fajardy I, Diot E, et al. Association between the IRF5 rs2004640 functional polymorphism and systemic sclerosis: a new perspective for pulmonary fibrosis. Arthritis Rheum 2009;60:225–33.

13. Gourh P, Agarwal SK, Divecha D, Assassi S, Paz G, Arora-Singh RK, et al. Polymorphisms in TBX21 and STAT4 increase the risk of systemic sclerosis: evidence of possible gene–gene interaction and alterations in Th1/Th2 cytokines. Arthritis Rheum 2009;60: 3794–806.

14. Dieude P, Guedj M, Wipff J, Ruiz B, Riemekasten G, Airo P, et al. NLRP1 influences the systemic sclerosis phenotype: a new clue for the contribution of innate immunity in systemic sclerosis-related fibrosing alveolitis pathogenesis. Ann Rheum Dis 2011;70: 668–74.

15. Bossini-Castillo L, Broen JC, Simeon CP, Beretta L, Vonk MC, Ortego-Centeno N, et al. A replication study confirms the association of TNFSF4 (OX40L) polymorphisms with systemic sclerosis in a large European cohort. Ann Rheum Dis 2011;70:638–41.

16. Dieude P, Guedj M, Truchetet ME, Wipff J, Revillod L, Riemekasten G, et al. Association of the CD226 Ser[307] variant with systemic sclerosis: evidence of a contribution of costimulation pathways in systemic sclerosis pathogenesis. Arthritis Rheum 2011;63:1097–105.

17. Gourh P, Agarwal SK, Martin E, Divecha D, Rueda B, Bunting H, et al. Association of the C8orf13-BLK region with systemic sclerosis in North-American and European populations. J Autoimmun 2010;34:155–62.

18. Dieude P, Guedj M, Wipff J, Ruiz B, Riemekasten G, Matucci-Cerinic M, et al. Association of the TNFAIP3 rs5029939 variant

with systemic sclerosis in the European Caucasian population. Ann Rheum Dis 2010;69:1958–64.

19. Tsuchiya N, Kawasaki A, Hasegawa M, Fujimoto M, Takehara K, Kawaguchi Y, et al. Association of STAT4 polymorphism with systemic sclerosis in a Japanese population. Ann Rheum Dis 2009;68:1375–6.

20. Ito I, Kawaguchi Y, Kawasaki A, Hasegawa M, Ohashi J, Hikami K, et al. Association of a functional polymorphism in the IRF5 region with systemic sclerosis in a Japanese population. Arthritis Rheum 2009;60:1845–50.

21. Ito I, Kawaguchi Y, Kawasaki A, Hasegawa M, Ohashi J, Kawamoto M, et al. Association of the FAM167A–BLK region with systemic sclerosis. Arthritis Rheum 2010;62:890–5.

22. Hasebe N, Kawasaki A, Ito I, Kawamoto M, Hasegawa M, Fujimoto M, et al. Association of UBE2L3 polymorphisms with diffuse cutaneous systemic sclerosis in a Japanese population. Ann Rheum Dis 2012;71:1259–60.

23. Zhou X, Lee JE, Arnett FC, Xiong M, Park MY, Yoo YK, et al. HLA–DPB1 and DPB2 are genetic loci for systemic sclerosis: a genome-wide association study in Koreans with replication in North Americans. Arthritis Rheum 2009;60:3807–14.

24. Suzuki A, Kochi Y, Okada Y, Yamamoto K. Insight from genome-wide association studies in rheumatoid arthritis and multiple sclerosis. FEBS Lett 2011;585:3627–32.

25. Plenge RM, Seielstad M, Padyukov L, Lee AT, Remmers EF, Ding B, et al. TRAF1-C5 as a risk locus for rheumatoid arthritis: a genomewide study. N Engl J Med 2007;357:1199–209.

26. Remmers EF, Plenge RM, Lee AT, Graham RR, Hom G, Behrens TW, et al. STAT4 and the risk of rheumatoid arthritis and systemic lupus erythematosus. N Engl J Med 2007;357:977–86.

27. Plenge RM, Cotsapas C, Davies L, Price AL, de Bakker PI, Maller J, et al. Two independent alleles at 6q23 associated with risk of rheumatoid arthritis. Nat Genet 2007;39:1477–82.

28. Thomson W, Barton A, Ke X, Eyre S, Hinks A, Bowes J, et al. Rheumatoid arthritis association at 6q23. Nat Genet 2007;39: 1431–3.

29. Begovich AB, Carlton VE, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander HC, et al. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. Am J Hum Genet 2004;75:330–7.

30. Diaz-Gallo LM, Gourh P, Broen J, Simeon C, Fonollosa V, Ortego-Centeno N, et al. Analysis of the influence of PTPN22 gene polymorphisms in systemic sclerosis. Ann Rheum Dis 2011; 70:454–62.

31. Okada Y, Terao C, Ikari K, Kochi Y, Ohmura K, Suzuki A, et al. Meta-analysis identifies nine new loci associated with rheumatoid arthritis in the Japanese population. Nat Genet 2012;44:511–6.

32. Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee.

Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum 1980;23:581–90.

33. LeRoy EC, Black C, Fleischmajer R, Jablonska S, Krieg T, Medsger TA Jr, et al. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. J Rheumatol 1988;15:202–5.

34. 1000 Genomes Project Consortium. A map of human genome variation from population-scale sequencing. Nature 2010;467: 1061–73.

35. Mimura Y, Ihn H, Jinnin M, Asano Y, Yamane K, Yazawa N, et al. Rheumatoid factor isotypes and anti-agalactosyl IgG antibodies in systemic sclerosis. Br J Dermatol 2004;151:803–8.

36. Santiago M, Baron M, Miyachi K, Fritzler MJ, Abu-Hakima M, Leclercq S, et al. A comparison of the frequency of antibodies to cyclic citrullinated peptides using a third generation anti-CCP assay (CCP3) in systemic sclerosis, primary biliary cirrhosis and rheumatoid arthritis. Clin Rheumatol 2008;27:77–83.

37. Yoshikawa F, Banno Y, Otani Y, Yamaguchi Y, Nagakura-Takagi Y, Morita N, et al. Phospholipase D family member 4, a trans-membrane glycoprotein with no phospholipase D activity, expression in spleen and early postnatal microglia. PLoS One 2010;5: e13932.

38. Otani Y, Yamaguchi Y, Sato Y, Furuichi T, Ikenaka K, Kitani H, et al. PLD4 is involved in phagocytosis of microglia: expression and localization changes of PLD4 are correlated with activation state of microglia. PLoS One 2011;6:e27544.

39. Stranger BE, Forrest MS, Dunning M, Ingle CE, Beazley C, Thorne N, et al. Relative impact of nucleotide and copy number variation on gene expression phenotypes. Science 2007;315: 848–53.

40. Cunninghame Graham DS, Morris DL, Bhangale TR, Criswell LA, Syvanen AC, Ronnblom L, et al. Association of NCF2, IKZF1, IRF8, IFIH1, and TYK2 with systemic lupus erythematosus. PLoS Genet 2011;7:e1002341.

41. Higgs BW, Liu Z, White B, Zhu W, White WI, Morehouse C, et al. Patients with systemic lupus erythematosus, myositis, rheumatoid arthritis and scleroderma share activation of a common type I interferon pathway. Ann Rheum Dis 2011;70:2029–36.

42. Whitfield ML, Finlay DR, Murray JI, Troyanskaya OG, Chi JT, Pergamenschikov A, et al. Systemic and cell type-specific gene expression patterns in scleroderma skin. Proc Natl Acad Sci U S A 2003;100:12319–24.

43. Lafyatis R, Kissin E, York M, Farina G, Viger K, Fritzler MJ, et al. B cell depletion with rituximab in patients with diffuse cutaneous systemic sclerosis. Arthritis Rheum 2009;60:578–83.

44. Smith V, Van Praet JT, Vandooren B, Van der Cruyssen B, Naeyaert JM, Decuman S, et al. Rituximab in diffuse cutaneous systemic sclerosis: an open-label clinical and histopathological study. Ann Rheum Dis 2010;69:193–7.

45. Hambleton S, Salem S, Bustamante J, Bigley V, Boisson-Dupuis S, Azevedo J, et al. IRF8 mutations and human dendritic-cell immunodeficiency. N Engl J Med 2011;365:127–38.

PLoS one

ACPA-Negative RA Consists of Two Genetically Distinct Subsets Based on RF Positivity in Japanese

Chikashi Terao[1,2]*, Koichiro Ohmura[1]*, Katsunori Ikari[3], Yuta Kochi[4], Etsuko Maruya[5], Masaki Katayama[1], Kimiko Yurugi[6], Kota Shimada[7], Akira Murasawa[8], Shigeru Honjo[9], Kiyoshi Takasugi[10], Keitaro Matsuo[11], Kazuo Tajima[11], Akari Suzuki[4], Kazuhiko Yamamoto[12], Shigeki Momohara[3], Hisashi Yamanaka[3], Ryo Yamada[2], Hiroo Saji[5], Fumihiko Matsuda[2,13,14], Tsuneyo Mimori[1]

1 Department of Rheumatology and Clinical Immunology, Kyoto University Graduate School of Medicine, Kyoto, Japan, 2 Center for Genomic Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan, 3 Institute of Rheumatology, Tokyo Women's Medical University, Tokyo, Japan, 4 Laboratory for Autoimmune Diseases, Center for Genomic Medicine, RIKEN, Yokohama, Japan, 5 HLA Laboratory, Kyoto, Japan, 6 Department of Transfusion Medicine and Cell Therapy, Kyoto University Hospital, Kyoto, Japan, 7 Department of Rheumatology, Sagamihara National Hospital, National Hospital Organization, Sagamihara, Japan, 8 Department of Rheumatology, Niigata Rheumatic Center, Niigata, Japan, 9 Rheumatoid Arthritis Center, Saiseikai Takaoka Hospital, Toyama, Japan, 10 Department of Internal Medicine, Center for Rheumatic Diseases, Dohgo Spa Hospital, Matsuyama, Japan, 11 Aichi Cancer Center Hospital and Research Institute, Nagoya, Japan, 12 Department of Allergy and Rheumatology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan, 13 CREST program, Japan Science and Technology Agency, Kawaguchi, Saitama, Japan, 14 Institut National de la Sante et de la Recherche Medicale (INSERM) Unite U852, Kyoto University Graduate School of Medicine, Kyoto, Japan

Abstract

HLA-DRB1, especially the shared epitope (SE), is strongly associated with rheumatoid arthritis (RA). However, recent studies have shown that SE is at most weakly associated with RA without anti-citrullinated peptide/protein antibody (ACPA). We have recently reported that ACPA-negative RA is associated with specific HLA-DRB1 alleles and diplotypes. Here, we attempted to detect genetically different subsets of ACPA-negative RA by classifying ACPA-negative RA patients into two groups based on their positivity for rheumatoid factor (RF). HLA-DRB1 genotyping data for totally 954 ACPA-negative RA patients and 2,008 healthy individuals in two independent sets were used. HLA-DRB1 allele and diplotype frequencies were compared among the ACPA-negative RF-positive RA patients, ACPA-negative RF-negative RA patients, and controls in each set. Combined results were also analyzed. A similar analysis was performed in 685 ACPA-positive RA patients classified according to their RF positivity. As a result, HLA-DRB1*04:05 and *09:01 showed strong associations with ACPA-negative RF-positive RA in the combined analysis (p = 8.8×10^{-6} and 0.0011, OR: 1.57 (1.28–1.91) and 1.37 (1.13–1.65), respectively). We also found that HLA-DR14 and the HLA-DR8 homozygote were associated with ACPA-negative RF-negative RA (p = 0.00022 and 0.00013, OR: 1.52 (1.21–1.89) and 3.08 (1.68–5.64), respectively). These association tendencies were found in each set. On the contrary, we could not detect any significant differences between ACPA-positive RA subsets. As a conclusion, ACPA-negative RA includes two genetically distinct subsets according to RF positivity in Japan, which display different associations with HLA-DRB1. ACPA-negative RF-positive RA is strongly associated with HLA-DRB1*04:05 and *09:01. ACPA-negative RF-negative RA is associated with DR14 and the HLA-DR8 homozygote.

Introduction

Rheumatoid arthritis (RA) is the most common cause of chronic arthritis worldwide and results in severe joint destruction [1]. Genetic and environmental factors have been shown to be associated with its onset [2–3]. Among the susceptibility genes to RA, HLA-DRB1 has been shown to be the strongest genetic determinant of RA susceptibility, and its association with RA susceptibility has been repeatedly shown to be independent of ethnicity [4–5]. A common amino acid sequence extending from the 70th to 74th in the HLA-DRβ chain, which is known as the

"shared epitope (SE)", is considered to be the reason for the association between HLA-DRB1 and RA, and the association between the SE and RA has been reported to be ethnicity-independent [6–8]. However, recent studies have shown that the SE is strongly associated with RA patients who have anti-citrullinated peptide/protein antibodies (ACPA), which is a highly specific marker of RA [9], but that it is not or only weakly associated with RA without ACPA [7,10–11]. Among the various HLA-DRB1 alleles, HLA-DR3 [12] and HLA-DR13 [13] were reported to be associated with ACPA-negative RA in populations of European descent, but these results were not confirmed in a

57