Exp. Anim. 58(2), 193–198, 2009

—Note—

Genetic Mapping Found Major QTLs for Antibody-Induced Glomerulonephritis in WKY Rats

Hiroyuki KOSE[1, 4)], Yoshikazu SADO[2)], Takahisa YAMADA[3)], and Kozo MATSUMOTO[1)]

[1)]Division for Animal Research Resources, Institute of Health Biosciences, The University of Tokushima Graduate School, Kuramoto-cho Tokushima 770-8503, [2)]Division of Immunology, Shigei Medical Research Institute, Okayama 701-0202, [3)]Laboratory of Animal Breeding and Genetics, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, and [4)]Present address: Department of Life Science, Division of Natural Sciences, International Christian University, Mitaka, Tokyo 181-8585, Japan

Abstract: Genetic bases of glomerulonephritis, a major cause of kidney dysfunction in humans and one of the most characteristic complications of autoimmune disorders such as Goodpasture syndrome, are complex. The Wistar-Kyoto (WKY) rat strain is well characterized for its susceptibility to autoantibodies against glomerular basement membrane (GBM), however the molecular mechanisms underlining the phenotype are largely unknown. Here we performed a whole genome scan using a backcross (BC) F_1 (WKY × DA) × WKY population, for which the DA rat is a nonsusceptible control strain. We found two significant QTLs on chromosomes 1 and 12, which were involved in elevated levels of proteinuria and kidney weight index, respectively. The relevance of these QTLs with the genetic factors involved in autoimmunity and renal disease is discussed.

Key words: glomerulonephritis, QTL analysis, WKY rat

Anti-glomerular basement membrane antibody-induced glomerulonephritis (anti-GBM nephritis) is an autoimmune disease caused by autoantibody against renal GBM. In about the half of the cases, pulmonary hemorrhage is superimposed, which is a pathogenic condition diagnosed as Goodpasture syndrome [9, 11]. Serum of patients with this disease contains autoantibodies against NC1 domains of type IV collagen alpha chains [α(IV)NC1] [3, 7, 10]. In humans, there are six genetically different isofroms of type IV collagen alpha chains [alpha1 to alpha6]. It has been suggested that among these isoforms, α3(IV)NC1 is the most likely autoantigen because serum from patients shows particularly high titers against the alpha3 isoform [12, 25]. The majority of GBM contains collagen triple fragments primarily composed of α3/α4/α5 subunits, along with a minor form of α1/α1/α2 triplets localized on the endothelial side of the GBM [21].

In order to elucidate the molecular nature of glomerulonephritis or Goodpasture syndrome, we developed experimental autoimmune models in the Wistar Kyoto (WKY) rat strain, which is uniquely susceptible to glomerulonephritis [13, 22]. Nephritis in this strain can be induced by injection of various forms of nephritogenic antigens, including solubilized GBM, a purified protein fraction of the α(IV)NC1 domain, synthetic peptides and

(Received 11 September 2008 / Accepted 15 January 2009)

Address corresponding: K. Matsumoto, Division of Animal Research Resources, Institute of Health Biosciences, The University of Tokushima Graduate School, 3 Kuramoto-cho, Tokushima 770-8503, Japan

recombinant proteins of α3(IV)NC1 or α4(IV)NC1 [18–20, 24, 28]. In clinical practice, these antigens are used as a diagnostic markers, suggesting that the nephritis of these models is similar to the human condition [7, 12, 26].

In contrast to antigen-induced, or actively induced nephritis, passive induction induces to the nephritis by injection of antibodies. We demonstrated that the polyclonal fractions obtained from the urine of nephritic rats, as well as monoclonal antibodies against GBM, can cause severe glomerulonephritis, suggesting that humoral immunity plays a crucial part in the disease's development [22, 23]. More recently, IgG2a and IgG2b subclasses were shown to be more potent agents than other subclasses against the same autoantigens [14].

Recently, Aitman *et al*. reported that multiple loci contribute to the nephritis-prone phenotypes of the WKY strain and the *Fcgr3* is one of the causative genes [1]. However, the molecular nature of anti-GBM nephritis has yet to be elucidated. Here we performed a whole genome scan to search for evidence of QTLs affecting susceptibility to nephritogenic agents in a backcrossed strain between WKY and nephritis-resistant DA rat strains.

Rat strains and animal procedures: WKY/NCrl inbred rats were purchased from Charles River Laboratories, Inc. (Boston, MA), and DA/Slc rats from Japan SLC, Inc. (Tokyo, Japan). The rats were housed in polycarbonate plastic cages containing wood shavings as bedding. They were given free access to drinking water and food. The animal experiments were conducted according to the guidelines of the Shigei Medical Research Institute for Laboratory Animal Experiments.

Genotyping and microsatellite markers: DNA isolation and polymerase chain reaction (PCR) amplification of microsatellite markers were performed as previously described [17]. We initially screened 251 markers to test their polymorphisms between WKY/NCrj and DA strains. We chose 112 markers separated by on average ca. 15.6 cM for QTL analysis.

Data analysis: Genetic markers were mapped relative to one another with the Map Manager QTX20b software package [16]. For each chromosome, the likelihood ratio statistic (LRS) significance values were calculated by simple interval mapping, then converted to LOD scores by dividing by 4.6 (2× the natural log of 10). Results were considered significant for LOD >3.3 or suggestive for >1.9, according to the guideline set by Lander and Krugliak [15]. Phenotypic comparisons of different genotypic groups were performed using Student's *t*-test or analysis of variance (ANOVA) with a post hoc test, Scheffe's test. (StatView sotfware for Macintosh, ver.5.0).

Injection of monoclonal antibody against GBM: Antibody injection and subsequent analysis was performed as previously reported [14]. Glomerulonephritis was induced in eight-week-old animals by a single injection of an anti-GBM monoclonal antibody, b35. The antibody was diluted with GIT culture medium (Wako Pure Chemical Industries, Osaka, Japan) containing 10% FBS. Using 1-ml disposable plastic injectors for tuberculin, we administered 500 μl of antibody to the abdominal cavity of rats under anesthesia. The rats had been fasted from the previous night in order to reduce the risk of possible infection due to needle penetration of the bowel during injection. Urine specimens were collected for fifteen hours (from 17:00 to 9:00) 5 days after the antibody injection, and were assayed for proteinuria by a modified method using 3% sulfosalicylic acid [24]. Body weight and kidney weight were measured 8 days after the injection. The kidney index was calculated from the kidney and body weights (percentage of kidney weight/body weight).

Mating scheme: We crossed female WKY rats with male DA rats to generate female F_1 progeny which, in turn, were crossed with WKY rats to make 118 female BC progeny. In our system only females were used because female proteinurea is more reproducible and consistent [24].

The WKY and DA rats showed clear differences in all nephritis-related phenotypes (Fig. 1 and Table 1). The level of proteinurea, which is an important indicator of kidney dysfunction, was inherited in a recessive manner. However, pairwise comparison between the DA and the F_1 showed minor, yet statistically significant differences (data not shown). This implies the presence of minor alleles which may contribute cooperatively to hamper the kidney function.

Kidney weight was inherited in an incompletely dominant manner, while the kidney weight index was

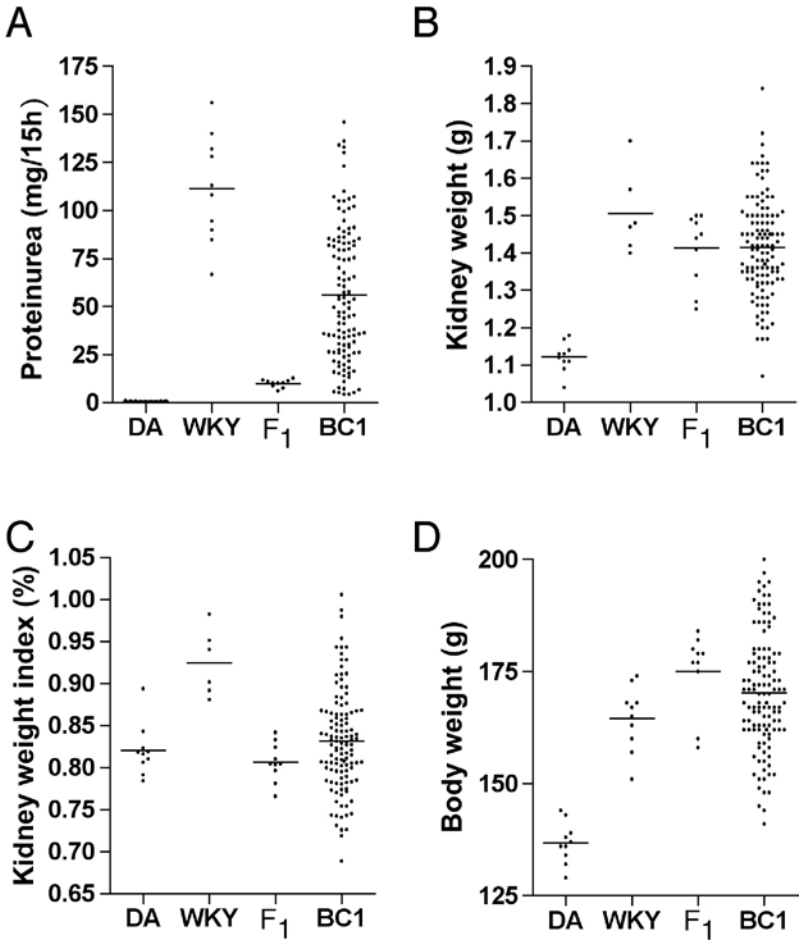

Fig. 1. Scatterplots of phenotypic characters. Panels display measurements from parental WKY and DA rats, and F_1 and 118 F_1 (WKY × DA) × WKY backcross (BC) rats. A) Proteinuria at 5 days after the injection of anti-GBM monoclonal antibody, b35. B–D) Kidney weight, the kidney weight index (kidney weight expressed as a percentage of body weight) and body weight. The weights were measured 8 days after antibody injection.

Table 1. Phenotypic characteristics of parental strains, F_1 progeny, and BC progeny

	DA (n=10)	WKY (n=10)	F_1 progeny (n=10)	BC progeny (n=118)
Proteinurea (mg/15 h)	1.2 ± 0.4	111.3 ± 8.8**	10.0 ± 0.6††	56.0 ± 3.2**
Kidney weight (g)	1.12 ± 0.013	1.51 ± 0.046**	1.41 ± 0.030**	1.41 ± 0.012**
Kidney weight index (%)[a)]	0.82 ± 0.01	0.93 ± 0.02*	0.81 ± 0.01†	0.83 ± 0.01
Body weight (g)	136.8 ± 1.5	164.6 ± 2.2**	175.1 ± 2.8**	170.3 ± 1.2**

[a)]Kidney weight index is the percentage of kidney weight/body weight. *P<0.01, ** P<0.0001 vs DA rats. $^{\dagger}P$<0.01, $^{\dagger\dagger}P$<0.0001 vs WKY rats.

inherited in a recessive manner. The different inheritance patterns suggest that these two traits are inherited under different genetic controls. Since body weight significantly differs between the parental strains, we decided that it was more appropriate to assess the kidney weight index rather than the kidney weight.

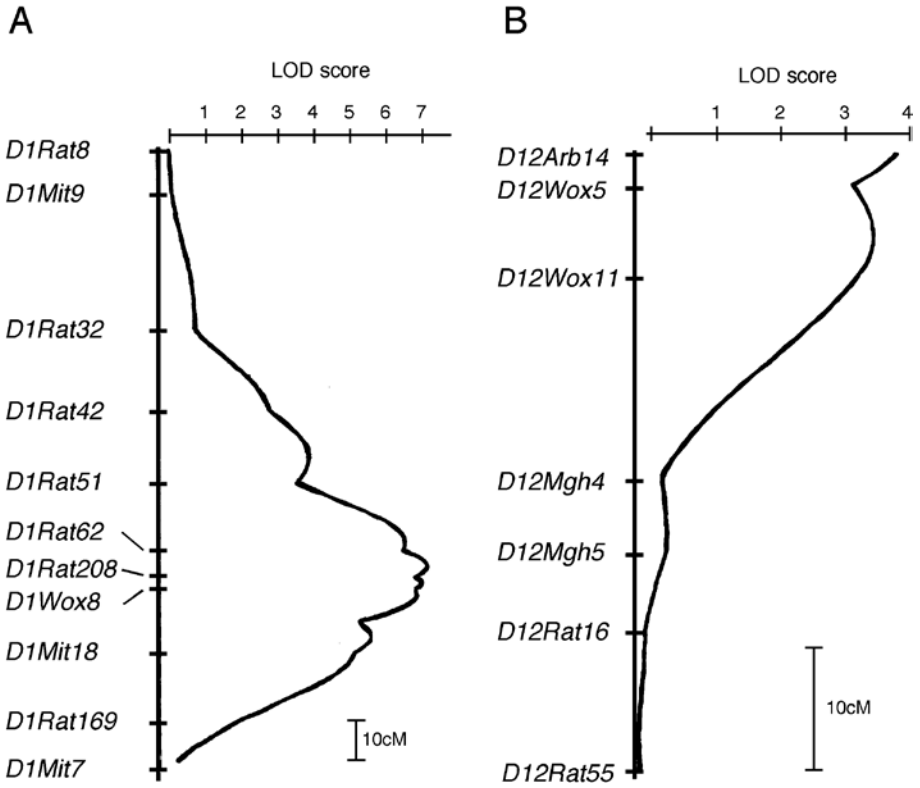

Fig. 2. Scans for lod scores for linkage. A) Urinary protein (chromosome 1). B) Kidney weight index (chromosome 12). The ordinate labels indicate microsatellite markers used for the linkage analysis, and map distances in centimorgans determined by the Kosambi map function are presented.

Table 2. Comparison of means of traits for BC progeny based on genotypes at *Again* loci

Locus	Chromosome	Phenotype	Genotype		*P*
			DA/WKY	WKY/WKY	
Again1	1	Proteinuria (mg/15 h)	39.5 ± 3.7	72.6.0 ± 4.2	<0.0001
n			59	59	
Again2	12	Kidney weight index	0.812 ± 0.07	0.856 ± 0.08	<0.0001
n			65	53	

Animals were grouped according to the genotypes of the markers (*D1Rat208* and *D12Arb14* for *Again1* and *2*, respectively) closest to the maximum LOD scores at each locus. *P* values are from Student's *t*-test.

To identify QTL(s) affecting susceptibility to anti-GBM antibody-induced glomerulonephritis, we carried out a total genome scan on the BC progeny using 112 informative microsatellite markers. Interval mapping using QTX software revealed that there were two significant QTLs (Fig. 2). A major locus, designated as *Again1* (anti-GBM antibody-induced glomerulonephritis) affecting urinary protein levels, is localized near *D1Rat208*, with a LOD score of 7.1. The inheritance pattern was consistent with WKY alleles acting to increase the urinary protein (Table 2). *Again1* was also shown to affect the kidney weight index, though the LOD score was only suggestive (data not shown).

A second locus was found on chromosome 12. This locus, near *D12Arb14*, was designated as *Again2*. It was linked to the kidney weight index with a maximum LOD

score of 3.7 (Fig. 2). A WKY allele was implied to contribute to nephritis (Table 2). Furthermore, *Again2* showed a suggestive LOD score for urinary protein. In WKY rats, there is a strong correlation between the degree of proteinuria and kidney weight, presumably because the ailing kidney tends to show edematous swelling. Accordingly, it is possible that a QTL responsible for the kidney weight index is also involved in kidney function or vice versa. Finally, in spite of the size difference between the parental strains, we were unable to detect a QTL for the body weight.

It has been many years since the discovery of the target antigen of Goodpasture syndrome. Much evidence has accumulated to indicate that autoantibody plays a crucial role in the pathogenesis of glomerulonephritis, however, understanding what external stimuli induce the autoantibody production and how the antibody aggravates and/or prolongs the inflammatory response remains an important challenge from the clinical perspective. This is mostly because a suitable animal model was not available until recently. Injection of either GBM antigen or anti-GBM antibody mainly results in only partial reproduction of the pathological features [6, 12, 27]. Therefore, the prominent susceptibility of the WKY rat to nephrotoxic stimuli provides a unique model to study the genetic components of anti-GBM nephritis.

In the current study, we identified two significant QTLs influencing levels of proteinuria and kidney weight index. Consistent with recently reported linkage analyses that were performed on the same strain [1, 2], we showed that there are multiple loci that contribute to the disease development. Yet, interestingly, neither *Again1* nor *2* was reported in this previous study. The discrepancy may be explained at least in part by the mating scheme (backcross vs intercross), the mating partner (DA vs Lewis), the sex and perhaps by different experimental procedure or some unknown environmental factors. However, we suggest that the most significant difference between the two studies is the nephrotoxic agents. In our study, the agent was a rat monoclonal antibody derived from the same species; therefore, it presumably better reproduced the pathophysiology of autoimmune disorder.

Again 1 and *2* are localized within approximately 23 and 15 cM in one-lod support intervals. The human genome regions homologous to these QTL intervals are 10q26 for *Again1* and 7p22 for *Again2*. However, these regions do not correspond to the HLA locus that was shown to have a strong association with the syndrome in humans, or the α3(IV)NC1 locus [8]. As to the mechanism of inflammation subsequent to antibody injection, it is widely accepted that activated macrophages play a central role in crescentic glomerulonephritis [4]. From this perspective, a couple of candidate genes were found in the *Again1* region including the gene encoding *Interleukin-4 receptor alpha chain precursor* (*IL4ra*). IL-4 has been shown to ameliorate crescentic glomerulonephritis in WKY rats [5]. Furthermore, one of the key functions of this cytokine is to inhibit antibody isotype switching towards the IgG2a subclass. Our previous study demonstrated that IgG2a and IgG2b subclass antibodies possess more nephritogenic activity than the IgG1 subclass. Thus, it is possible that the WKY rat allele of this gene may contribute to anti-GBM nephritis development by suppressing the IL4 signaling pathway. Similarly, we think that the *Interleukin-3 receptor alpha chain precursor* (*IL3ra*) gene is a good candidate gene in the *Again2* locus, given the role of IL3 in inflammatory response.

Future fine mapping analyses using congenic strains are being planned. They will facilitate a better understanding of the complex interplay among immunological systems with regard to the development of anti-GBM nephritis.

Acknowledgment(s)

This study was supported in part by a grant from the National BioResource Project (NBRP) for the Rat in Japan (K.M.) and from the Ministry of Education, Culture, Sports, Science and Technology of Japan (H.K. and K.M). We thank Takako Koizumi and Chieko Takahashi for technical assistance in breeding rat strains.

References

1. Aitman, T.J., Dong, R., Vyse, T.J., Norsworthy, P.J., Johnson, M.D., Smith, J., Mangion, J., Roberton-Lowe, C., Marshall, A.J., Petretto, E., Hodges, M.D., Bhangal, G., Patel, S.G., Sheehan-Rooney, K., Duda, M., Cook, P.R., Evans, D.J., Domin, J., Flint, J., Boyle, J.J., Pusey, C.D., and Cook, H.T.

2006. *Nature* 439: 851–855.
2. Behmoaras, J., Bhangal, G., Smith, J., McDonald, K., Mutch, B., Lai, P.C., Domin, J., Game, L., Salama, A., Foxwell, B.M., Pusey, C.D., Cook, H.T., and Aitman, T.J. 2008. *Nat. Genet*. 40: 553–559.
3. Butkowski, R.J., Langeveld, J.P., Wieslander, J., Hamilton, J., and Hudson, B.G. 1987. *J. Biol. Chem*. 262: 7874–7877.
4. Cattell, V. 1994. *Kidney Int*. 45: 945–952.
5. Cook, H.T., Singh, S.J., Wembridge, D.E., Smith, J., Tam, F.W., and Pusey, C.D. 1999. *Kidney Int*. 55: 1319–1326.
6. Couser, W.G., Darby, C., Salant, D.J., Adler, S., Stilmant, M.M., and Lowenstein, L.M. 1985. *Am. J. Physiol*. 249: F241–250.
7. Dehan, P., Weber, M., Zhang, X., Reeders, S.T., Foidart, J.M., and Tryggvason, K. 1996. *Nephrol. Dial. Transplant*. 11: 2215–2222.
8. Fisher, M., Pusey, C.D., Vaughan, R.W., and Rees, A.J. 1997. *Kidney Int*. 51: 222–229.
9. Hudson, B.G., Kalluri, R., Gunwar, S., Noelken, M.E., Mariyama, M., and Reeders, S.T. 1993. *Kidney Int*. 43: 135–139.
10. Hudson, B.G., Reeders, S.T., and Tryggvason, K. 1993. *J. Biol. Chem*. 268: 26033–26036.
11. Hudson, B.G., Tryggvason, K., Sundaramoorthy, M., and Neilson, E.G. 2003. *N. Engl. J. Med*. 348: 2543–2556.
12. Kalluri, R., Wilson, C.B., Weber, M., Gunwar, S., Chonko, A.M., Neilson, E.G., and Hudson, B.G. 1995. *J. Am. Soc. Nephrol*. 6: 1178–1185.
13. Kawasaki, K., Yaoita, E., Yamamoto, T., and Kihara, I. 1992. *Kidney Int*. 41: 1517–1526.
14. Kohda, T., Okada, S., Hayashi, A., Kanzaki, S., Ninomiya, Y., Taki, M., and Sado, Y. 2004. *Kidney Int*. 66: 177–186.
15. Lander, E. and Kruglyak, L. 1995. *Nat. Genet*. 11: 241–247.
16. Manly, K.F., Cudmore, R.H. Jr., and Meer, J.M. 2001. *Mamm. Genome* 12: 930–932.
17. Moralejo, D.H., Wei, S., Wei, K., Weksler-Zangen, S., Koike, G., Jacob, H.J., Hirashima, T., Kawano, K., Sugiura, K., Sasaki, Y., Ogino, T., Yamada, T., and Matsumoto, K. 1998. *Proc. Assoc. Am. Physicians*. 110: 545–558.
18. Ryan, J.J., Reynolds, J., Norgan, V.A., and Pusey, C.D. 2001. *Nephrol. Dial. Transplant*. 16: 253–261.
19. Sado, Y., Boutaud, A., Kagawa, M., Naito, I., Ninomiya, Y., and Hudson, B.G. 1998. *Kidney Int*. 53: 664–671.
20. Sado, Y., Kagawa, M., Naito, I., and Okigaki, T. 1991. *Virchows Arch. B. Cell Pathol. Incl. Mol. Pathol*. 60: 345–351.
21. Sado, Y., Kagawa, M., Naito, I., Ueki, Y., Seki, T., Momota, R., Oohashi, T., and Ninomiya, Y. 1998. *J. Biochem*. 123: 767–776.
22. Sado, Y., Kagawa, M., Rauf, S., Naito, I., Moritoh, C., and Okigaki, T. 1992. *J. Pathol*. 168: 221–227.
23. Sado, Y., Naito, I., and Okigaki, T. 1989. *J. Pathol*. 158: 325–332.
24. Sado, Y., Watanabe, K., Okigaki, T., Takamiya, H., and Seno, S. 1984. *Biochim. Biophys. Acta* 798: 96–102.
25. Saus, J., Wieslander, J., Langeveld, J.P., Quinones, S., and Hudson, B.G. 1988. *J. Biol. Chem*. 263: 13374–13380.
26. Saxena, R., Bygren, P., Rasmussen, N., and Wieslander, J. 1991. *Nephrol. Dial. Transplant*. 6: 389–397.
27. Steblay, R.W. 1979. *Am. J. Pathol*. 97: 649–652.
28. Sugihara, K., Sado, Y., Ninomiya, Y., and Wada, H. 1996. *J. Pathol*. 178: 352–358.