cells and PDCs (47). Very recently, epidermal keratinocytes damaged by UV light exposure were shown to release self-noncoding RNA by which TLR-3 was ligated, leading to an alteration in immune responses (48). In addition, self-nucleic acids released after skin injury have been shown to trigger TLR-7/TLR-9 activation and to promote PDC infiltrates and the production of type I IFN (49,50). In this regard, we speculate that the continuous release of self-RNA from apoptotic epidermal cells after injury or solar damage may trigger homeostatic TLR-7 activation, leading to systemic autoimmune disease. Patients with SLE are photosensitive, and this effect was also reproduced in imiquimod-treated mice. It is noteworthy that the skin where imiquimod was applied showed marked photosensitivity, suggesting that locally activated TLR-7 in the skin might contribute to an acute inflammatory response to UV irradiation. In conclusion, the present study not only provides a novel protocol for generating a lupus model in WT mice but also highlights the skin as the primary organ where TLR-7 signaling leads to systemic autoimmunity, as in SLE.

ACKNOWLEDGMENTS

We thank Dr. M. Matsumoto for performing histologic assessment, Ms T. Nagayama, Mr. K. Sato, and Dr. K. Ogura for technical assistance, and Dr. K. Miyake for helpful discussion.

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. Sano 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. Sano.

Acquisition of data. Yokogawa, Takaishi, Nakajima, Kamijima, Fujimoto, Kataoka.

Analysis and interpretation of data. Terada, Sano.

REFERENCES

1. Kotzin BL. Systemic lupus erythematosus. Cell 1996;85:303–6.
2. Christensen SR, Shlomchik MJ. Regulation of lupus-related autoantibody production and clinical disease by Toll-like receptors. Semin Immunol 2007;19:11–23.
3. Ewald SE, Barton GM. Nucleic acid sensing Toll-like receptors in autoimmunity. Curr Opin Immunol 2011;23:3–9.
4. Gilliet M, Lande R. Antimicrobial peptides and self-DNA in autoimmune skin inflammation. Curr Opin Immunol 2008;20:401–7.
5. Pascual V, Farkas L, Banchereau J. Systemic lupus erythematosus: all roads lead to type I interferons. Curr Opin Immunol 2006;18:676–82.
6. Berland R, Fernandez L, Kari E, Han JH, Lomakin I, Akira S, et al. Toll-like receptor 7-dependent loss of B cell tolerance in pathogenic autoantibody knockin mice. Immunity 2006;25:429–40.
7. Pasare C, Medzhitov R. Control of B-cell responses by Toll-like receptors. Nature 2005;438:364–8.
8. Subramanian S, Tus K, Li QZ, Wang A, Tian XH, Zhou J, et al. A Tlr7 translocation accelerates systemic autoimmunity in murine lupus. Proc Natl Acad Sci U S A 2006;103:9970–5.
9. Pisitkun P, Deane JA, Difilippantonio MJ, Tarasenko T, Satterthwaite AB, Bolland S. Autoreactive B cell responses to RNA-related antigens due to TLR7 gene duplication. Science 2006;312:1669–72.
10. Santiago-Raber ML, Baudino L, Izui S. Emerging roles of TLR7 and TLR9 in murine SLE. J Autoimmun 2009;33:231–8.
11. Santiago-Raber ML, Kikuchi S, Borel P, Uematsu S, Akira S, Kotzin BL, et al. Evidence for genes in addition to Tlr7 in the Yaa translocation linked with acceleration of systemic lupus erythematosus. J Immunol 2008;181:1556–62.
12. Berghofer B, Frommer T, Haley G, Fink L, Bein G, Hackstein H. TLR7 ligands induce higher IFN-α production in females. J Immunol 2006;177:2088–96.
13. Anders HJ. Pseudoviral immunity: a novel concept for lupus. Trends Mol Med 2009;15:553–61.
14. Amano H, Amano E, Moll T, Marinkovic D, Ibnou-Zekri N, Martinez-Soria E, et al. The Yaa mutation promoting murine lupus causes defective development of marginal zone B cells. J Immunol 2003;170:2293–301.
15. Bolland S, Yim YS, Tus K, Wakeland EK, Ravetch JV. Genetic modifiers of systemic lupus erythematosus in FcγRIIB$^{-/-}$ mice. J Exp Med 2002;195:1167–74.
16. Deane JA, Pisitkun P, Barrett RS, Feigenbaum L, Town T, Ward JM, et al. Control of Toll-like receptor 7 expression is essential to restrict autoimmunity and dendritic cell proliferation. Immunity 2007;27:801–10.
17. Christensen SR, Shupe J, Nickerson K, Kashgarian M, Flavell RA, Shlomchik MJ. Toll-like receptor 7 and TLR9 dictate autoantibody specificity and have opposing inflammatory and regulatory roles in a murine model of lupus. Immunity 2006;25:417–28.
18. Nickerson KM, Christensen SR, Shupe J, Kashgarian M, Kim D, Elkon K, et al. TLR9 regulates TLR7- and MyD88-dependent autoantibody production and disease in a murine model of lupus. J Immunol 2010;184:1840–8.
19. Reeves WH, Lee PY, Weinstein JS, Satoh M, Lu L. Induction of autoimmunity by pristane and other naturally occurring hydrocarbons. Trends Immunol 2009;30:455–64.
20. Rolink AG, Pals ST, Gleichmann E. Allosuppressor and allohelper T cells in acute and chronic graft-vs.-host disease. II. F_1 recipients carrying mutations at H-2K and/or I-A. J Exp Med 1983;157:755–71.
21. Kool M, van Loo G, Waelput W, De Prijck S, Muskens F, Sze M, et al. The ubiquitin-editing protein A20 prevents dendritic cell activation, recognition of apoptotic cells, and systemic autoimmunity. Immunity 2011;35:82–96.
22. Taylor PR, Martinez-Pomares L, Stacey M, Lin HH, Brown GD, Gordon S. Macrophage receptors and immune recognition. Annu Rev Immunol 2005;23:901–44.
23. Ishikawa S, Sato T, Abe M, Nagai S, Onai N, Yoneyama H, et al. Aberrant high expression of B lymphocyte chemokine (BLC/CXCL13) by C11b$^+$CD11c$^+$ dendritic cells in murine lupus and preferential chemotaxis of B1 cells towards BLC. J Exp Med 2001;193:1393–402.
24. Azeredo da Silveira S, Kikuchi S, Fossati-Jimack L, Moll T, Saito T, Verbeek JS, et al. Complement activation selectively potentiates the pathogenicity of the IgG2b and IgG3 isotypes of a high affinity anti-erythrocyte autoantibody. J Exp Med 2002;195:665–72.
25. Savitsky DA, Yanai H, Tamura T, Taniguchi T, Honda K. Contribution of IRF5 in B cells to the development of murine

SLE-like disease through its transcriptional control of the IgG2a locus. Proc Natl Acad Sci U S A 2010;107:10154–9.
26. Banchereau J, Pascual V. Type I interferon in systemic lupus erythematosus and other autoimmune diseases. Immunity 2006; 25:383–92.
27. Gilliet M, Conrad C, Geiges M, Cozzio A, Thurlimann W, Burg G, et al. Psoriasis triggered by Toll-like receptor 7 agonist imiquimod in the presence of dermal plasmacytoid dendritic cell precursors. Arch Dermatol 2004;140:1490–5.
28. Blanco P, Palucka AK, Gill M, Pascual V, Banchereau J. Induction of dendritic cell differentiation by IFN-α in systemic lupus erythematosus. Science 2001;294:1540–3.
29. Robak E, Smolewski P, Wozniacka A, Sysa-Jedrzejowska A, Robak T. Clinical significance of circulating dendritic cells in patients with systemic lupus erythematosus. Mediators Inflamm 2004;13:171–80.
30. Melen K, Ronni T, Broni B, Krug RM, von Bonsdorff CH, Julkunen I. Interferon-induced Mx proteins form oligomers and contain a putative leucine zipper. J Biol Chem 1992;267: 25898–907.
31. Klinkhoff AV, Thompson CR, Reid GD, Tomlinson CW. M-mode and two-dimensional echocardiographic abnormalities in systemic lupus erythematosus. JAMA 1985;253:3273–7.
32. Christensen SR, Kashgarian M, Alexopoulou L, Flavell RA, Akira S, Shlomchik MJ. Toll-like receptor 9 controls anti-DNA autoantibody production in murine lupus. J Exp Med 2005;202:321–31.
33. Fukui R, Saitoh S, Kanno A, Onji M, Shibata T, Ito A, et al. Unc93B1 restricts systemic lethal inflammation by orchestrating Toll-like receptor 7 and 9 trafficking. Immunity 2011;35:69–81.
34. Fukui R, Miyake K. Controlling systems of nucleic acid sensing-TLRs restrict homeostatic inflammation. Exp Cell Res 2012;318: 1461–6.
35. Baccala R, Hoebe K, Kono DH, Beutler B, Theofilopoulos AN. TLR-dependent and TLR-independent pathways of type I interferon induction in systemic autoimmunity. Nat Med 2007;13: 543–51.
36. Blasius AL, Giurisato E, Cella M, Schreiber RD, Shaw AS, Colonna M. Bone marrow stromal cell antigen 2 is a specific marker of type I IFN-producing cells in the naive mouse, but a promiscuous cell surface antigen following IFN stimulation. J Immunol 2006;177:3260–5.
37. Takagi H, Fukaya T, Eizumi K, Sato Y, Sato K, Shibazaki A, et al. Plasmacytoid dendritic cells are crucial for the initiation of inflammation and T cell immunity in vivo. Immunity 2011;35:958–71.
38. Baenziger S, Heikenwalder M, Johansen P, Schlaepfer E, Hofer U, Miller RC, et al. Triggering TLR7 in mice induces immune activation and lymphoid system disruption, resembling HIV-mediated pathology. Blood 2009;113:377–88.
39. Damm J, Wiegand F, Harden LM, Gerstberger R, Rummel C, Roth J. Fever, sickness behavior, and expression of inflammatory genes in the hypothalamus after systemic and localized subcutaneous stimulation of rats with the Toll-like receptor 7 agonist imiquimod. Neuroscience 2012;201:166–83.
40. Burns CA, Brown MD. Imiquimod for the treatment of skin cancer. Dermatol Clin 2005;23:151–64, vii.
41. Van der Fits L, Mourits S, Voerman JS, Kant M, Boon L, Laman JD, et al. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. J Immunol 2009;182: 5836–45.
42. Nestle FO, Conrad C, Tun-Kyi A, Homey B, Gombert M, Boyman O, et al. Plasmacytoid predendritic cells initiate psoriasis through interferon-α production. J Exp Med 2005;202:135–43.
43. Farkas L, Beiske K, Lund-Johansen F, Brandtzaeg P, Jahnsen FL. Plasmacytoid dendritic cells (natural interferon-α/β-producing cells) accumulate in cutaneous lupus erythematosus lesions. Am J Pathol 2001;159:237–43.
44. Wollenberg A, Wagner M, Gunther S, Towarowski A, Tuma E, Moderer M, et al. Plasmacytoid dendritic cells: a new cutaneous dendritic cell subset with distinct role in inflammatory skin diseases. J Invest Dermatol 2002;119:1096–102.
45. Barr KL, Konia TH, Fung MA. Lupus erythematosus-like imiquimod reaction: a diagnostic pitfall. J Cutan Pathol 2011;38: 346–50.
46. Baudino L, Yoshinobu K, Morito N, Santiago-Raber ML, Izui S. Role of endogenous retroviruses in murine SLE. Autoimmun Rev 2010;10:27–34.
47. Meller S, Winterberg F, Gilliet M, Muller A, Lauceviciute I, Rieker J, et al. Ultraviolet radiation–induced injury, chemokines, and leukocyte recruitment: an amplification cycle triggering cutaneous lupus erythematosus. Arthritis Rheum 2005;52:1504–16.
48. Bernard JJ, Cowing-Zitron C, Nakatsuji T, Muehleisen B, Muto J, Borkowski AW, et al. Ultraviolet radiation damages self noncoding RNA and is detected by TLR3. Nat Med 2012;18:1286–90.
49. Gregorio J, Meller S, Conrad C, Di Nardo A, Homey B, Lauerma A, et al. Plasmacytoid dendritic cells sense skin injury and promote wound healing through type I interferons. J Exp Med 2010;207: 2921–30.
50. Guiducci C, Tripodo C, Gong M, Sangaletti S, Colombo MP, Coffman RL, et al. Autoimmune skin inflammation is dependent on plasmacytoid dendritic cell activation by nucleic acids via TLR7 and TLR9. J Exp Med 2010;207:2931–42.

J Infect Chemother 21 (2015) 62–64

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic

Case report

A case of secondary syphilis demonstrating nephrotic syndrome and a solitary intrahepatic mass in a human immunodeficiency virus-1-infected patient

Hiroshi Koganemaru [a, *], Shigemi Hitomi [a], Hirayasu Kai [b], Kunihiro Yamagata [b]

[a] Department of Infectious Diseases, Faculty of Medicine, University of Tsukuba, Japan
[b] Department of Nephrology, Faculty of Medicine, University of Tsukuba, Japan

ARTICLE INFO

Article history:
Received 15 May 2014
Received in revised form
17 July 2014
Accepted 18 July 2014
Available online 7 August 2014

Keywords:
Secondary syphilis
Nephrotic syndrome
Human immunodeficiency virus
Intrahepatic mass

ABSTRACT

A 37-year-old, human immunodeficiency virus-1-infected Japanese man was referred because of nephrotic syndrome following emergence of generalized skin rash. Serological tests for syphilis turned out to be positive within ten months of the referral. Abdominal echography incidentally revealed a solitary intrahepatic mass without a detectable blood flow in segment 7. The patient's signs and symptoms, as well as the intrahepatic mass, resolved promptly after administration of amoxicillin. We consider that, in the present case, secondary syphilis caused the nephrotic syndrome and the intrahepatic mass, both of which have rarely been reported to date.

1. Introduction

Syphilis is a complex systemic disease caused by infection of *Treponema pallidum*, usually acquired through sexual contact or transmitted from mother to baby. Acquired syphilis is generally divided into four stages: primary, secondary, latent, and tertiary diseases. Each stage is characterized with typical clinical and laboratory presentations [1]. However, recent prevalence of human immunodeficiency virus (HIV) infection, another sexually transmitted disease, has altered the epidemiology of syphilis, including more severe manifestation in early stages and more rapid progression to late stages [2].

Presentation of diverse symptoms due to multisystem involvement is a characteristic of secondary syphilis. Skin rash, condylomata lata, mucocutaneous lesions, and generalized lymphadenopathy are those commonly demonstrated in this stage [3,4]. Rare manifestations include hepatitis [5,6], proctitis [7], meningitis [8], and arthritis [9]. Nephrotic syndrome is a complication infrequently reported in patients with secondary syphilis [10,11]. A study conducted in the pre-penicillin era showed that 0.28% of patients with early syphilis developed nephrotic syndrome [12]. In contrast, only a few cases of syphilitic nephrosis have been reported among patients with HIV infection [13,14]. Here, we report a case of an HIV-1-infected patient developing nephrotic syndrome associated with secondary syphilis. In addition, the patient demonstrated a solitary intrahepatic mass, an extremely rare manifestation in secondary syphilis.

2. Case report

A 37-year-old, bisexual Japanese man was referred because of edema on bilateral lower legs lasting for four months and exertional dyspnea worsening since one month before. He had a diagnosis of HIV-1 infection ten months before the referral, at which time both non-treponemal and treponemal serological tests were negative, but did not receive antiretroviral therapy. Although he denied any sexual contacts from the diagnosis, skin rash on the palms and the soles emerged two months before the referral. He did not note any previous symptoms indicating renal impairment.

On referral, the patient appeared unwell and complained of general fatigue, anorexia, and nonproductive cough. Abdominal pain, diarrhea, and nausea were denied. His axillary temperature was 37.4 °C and blood pressure was 152/95 mmHg. Physical examination revealed macular rash over the trunk, palms, and soles, painless lymphadenopathy in the bilateral axillary and inguinal regions, pretibial pitting edema on both legs, and systolic murmurs.

* Corresponding author. Department of Infectious Diseases, Faculty of Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8577, Japan. Tel.: +81 29 853 3210; fax: +81 29 853 3479.
E-mail address: hkogane-tkb@umin.org (H. Koganemaru).

http://dx.doi.org/10.1016/j.jiac.2014.07.013
1341-321X/

No remarkable finding was manifested through abdominal examination. Blood tests showed normocytic anemia (erythrocyte: $2.75 \times 10^6/\mu L$, hemoglobin: 8.0 g/dL, mean corpuscular volume: 89.8 fL), hypoalbuminemia (2.2 g/dL), hypergammaglobulinemia (IgG: 3613 mg/dL, IgM: 496 mg/dL), and elevation of serum creatinine (1.71 mg/dL). Creatinine clearance estimated with Cockcroft and Gault's equation was 58.6 mL/min. Total cholesterol was within a normal range (178 mg/dL). The CD4+ lymphocyte count and the plasma HIV-1 viral load were 403/μL and 1.8×10^5 copy/mL, respectively. Both rapid plasma reagin (RPR; 716.8 RPR unit) and treponema pallidum latex agglutination were positive. Anti-proteinase 3 antineutrophil cytoplasmic antibodies (ANCA) and anti-myeloperoxidase ANCA were negative. Urinalysis with a dipstick test showed marked proteinuria and hematuria. The protein/creatinine ratio in the urine was 5.6 g/g creatinine. Abdominal echography demonstrated enlargement of bilateral kidneys with echogenic cortices and a hypoechoic mass without a detectable blood flow, $3.5 \times 2.4 \times 2.0$ cm in diameter, in segment 7 of the liver (Fig. 1A). Histopathological examinations of the kidneys and the lymph nodes were declined.

Under diagnosis of nephrotic syndrome with hypertension and secondary syphilis, oral furosemide (20 mg per day), release-controlled nifedipine (10 mg per day) and valsartan (40 mg per day) were given along with counseling on diet for restricting protein and sodium intakes. Oral amoxicillin (1 g per day) was also administered for four weeks because benzathine penicillin G, commonly used for treatment of early syphilis worldwide, is unavailable in Japan. General fatigue, skin rash, edema, and dyspnea improved and the size of swollen lymph nodes reduced within two weeks of the treatments. The Jarisch–Herxheimer reaction did not occur. The intrahepatic mass on echography disappeared almost completely after six weeks (Fig. 1B). Serum albumin and creatinine levels (4.0 g/dL and 1.02 mg/dL, respectively), as well as estimated creatinine clearance (95.4 mL/min), were normalized after ten weeks. However, daily quantities of urine protein excretion, measured using Urinemate® P (Sumitomo Bakelite, Tokyo, Japan), remained between 1.8 and 6.1 g until 23 weeks. Antiretroviral therapy with raltegravir (800 mg per day) and abacavir/lamivudine (600 mg/300 mg per day) was initiated after seven months. At a follow-up after 16 months, the serum RPR level and the daily quantity of urine protein excretion decreased to 4.6 RPR unit and 0.74 g, respectively.

3. Discussion

In the present case, positive conversion of syphilitic serologies and emergence of generalized skin rash showed that the patient was newly infected with syphilis, which had advanced to the secondary stage on referral. In addition to secondary syphilis, the patient had HIV infection, another potential cause of nephrosis [15]. Histopathological examination of the kidney may differentiate the etiology: membranous glomerulonephropathy is commonly found in those caused by syphilis [16,17] whereas focal segmental glomerulosclerosis by HIV infection [18,19]. In the present case, renal biopsy was declined. Then, histopathological diagnosis was unavailable. However, the patient's complaints, renal dysfunction, and hypoalbuminemia were rapidly improved after administration of amoxicillin and without treatment for HIV infection. Thus, we consider that, in the present case, the secondary syphilis was the principal cause of the nephrosis and HIV infection may have minimally, if at all, influenced the pathogenesis.

To our knowledge, there have been two reported cases of syphilitic nephrosis occurring in HIV-infected-patients. The first case was a 22-year-old homosexual man presenting pitting edema on the lower extremities and scrotum and a diffuse erythematous papulopustular rash (CD4+ lymphocyte count = 400/μL) [13]. Intravenous penicillin (20 mU per day) for 10 days improved the edema, hypoalbuminemia (1.2 g/dL to 2.7 g/dL), and proteinuria (7.3 g/day to 1.4 g/day). The other was a 30-year-old African-American man with chronic kidney disease, who was diagnosed with neurosyphilis at presentation (CD4+ lymphocyte count = 1/μL) [14]. After administration of intravenous ceftriaxone for 14 days, quantities of urine protein excretion decreased (4.5 g/day to 0.8 g/day) and serum creatinine levels returned to baseline (3 mg/dL to 1.8 mg/dL). It is unclear why only a few cases of syphilitic nephrosis have been reported among HIV-infected patients to date. One possibility is that immune dysregulation by HIV infection affects the frequency and severity of immune-mediated renal injury associated with syphilis. Another is that both syphilis and HIV infection may develop nephrotic syndrome so that precise differentiation of primary cause is difficult clinically.

In the present case, the daily quantity of urinary protein excretion on referral, estimated with the protein/creatinine ratio of a spot urine specimen [20], was approximately 5 g. Although the quantities gradually decreased, proteinuria persisted even 16 months after

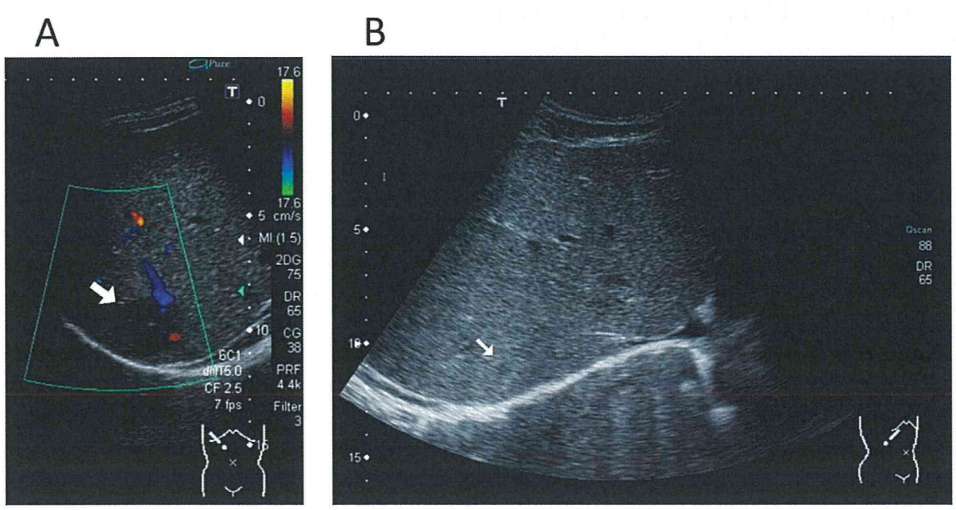

Fig. 1. Abdominal echograms taken on referral (A) and six weeks after antibiotic therapy was started (B). Arrows indicate an intrahepatic mass without a detectable blood flow ($3.5 \times 2.4 \times 2.0$ cm in diameter) in segment 7 of the liver (A), which disappeared almost completely after antibiotic therapy (B).

antibacterial treatment. Generally, a short course of antimicrobial therapy resolves proteinuria caused by syphilitic nephrosis within several months [16,21–23]. In contrast, a case report, published in the 1970s, described that it took 18 months to confirm disappearance of proteinuria [24]. Reasons for the persistence of proteinuria in certain cases are unclear. Fortunately, to our knowledge, patients with syphilitic nephrosis may not suffer from serious sequelae after appropriate antibacterial therapy has been given [13,17,21].

A solitary intrahepatic mass, another unique finding in the present case, was incidentally detected during intraabdominal examination with echography. The mass, although initially suspected of being malignant lymphoma or an abscess, promptly disappeared after administration of amoxicillin, suggesting that the lesion was benign and associated with amoxicillin-susceptible organisms. To our knowledge, three case reports have described intrahepatic benign masses that resolved after treatment of syphilis [25–27]. Histopathological examination of an intrahepatic benign mass by Mahto et al. demonstrated proliferation of young fibroblasts and blood vessels with heavy lymphoplasmacytic infiltrate, typical findings of inflammatory pseudotumor [26]. We speculate that the intrahepatic mass in the present case was due to similar inflammatory changes, possibly caused by *T. pallidum* infection.

Diagnosis of syphilis may be more challenging under the prevalence of HIV infection not only because of the intrinsic variety of clinical pictures but also modified presentations caused by HIV co-infection. Because syphilis in early stages is easily treatable with antibiotics even in patients with HIV co-infection, physicians should take care not to overlook this disease. It is uncertain whether the two unusual manifestations, nephrotic syndrome and an intrahepatic mass, were associated with underlying HIV infection in the present case. Further accumulation of similar cases is necessary to clarify the significance of these manifestations.

Conflict of interest

None.

Acknowledgment

We thank Prof. John A. Tokarz for his kind assistance in preparing this manuscript.

References

[1] Goh BT. Syphilis in adults. Sex Transm Infect 2005;81:448–52.
[2] Lynn WA, Lightman S. Syphilis and HIV: a dangerous combination. Lancet Infect Dis 2004;4:456–66.
[3] Lee V, Kinghorn G. Syphilis: an update. Clin Med 2008;8:330–3.
[4] Baughn RE, Musher DM. Secondary syphilitic lesions. Clin Microbiol Rev 2005;18:205–16.
[5] Crum-Cianflone N, Weekes J, Bavaro M. Syphilitic hepatitis among HIV-infected patients. Int J STD AIDS 2009;20:278–84.
[6] Mullick CJ, Liappis AP, Benator DA, Roberts AD, Parenti DM, Simon GL. Syphilitic hepatitis in HIV-infected patients: a report of 7 cases and review of the literature. Clin Infect Dis 2004;39. e100–5.
[7] Adachi E, Koibuchi T, Okame M, Sato H, Imai K, Shimizu S, et al. Case of secondary syphilis presenting with unusual complications: syphilitic proctitis, gastritis, and hepatitis. J Clin Microbiol 2011;49:4394–6.
[8] Strom T, Schneck SA. Syphilitic meningomyelitis. Neurology 1991;41:325–6.
[9] Kazlow PG, Beyer B, Brandeis G. Polyarthritis as the initial symptom of secondary syphilis: case report and review. Mt Sinai J Med 1989;56:65–7.
[10] Falls Jr WF, Ford KL, Ashworth CT, Carter NW. The nephrotic syndrome in secondary syphilis: report of a case with renal biopsy findings. Ann Intern Med 1965;63:1047–58.
[11] Braunstein GD, Lewis EJ, Galvanek EG, Hamilton A, Bell WR. The nephrotic syndrome associated with secondary syphilis: an immune deposit disease. Am J Med 1970;48:643–8.
[12] Hermann G, Marr WL. Clinical syphilitic nephropathies. A study of new cases and a survey of reported cases. Am J Syph Neurol 1935;19:1–29.
[13] Kusner DJ, Ellner JJ. Syphilis–a reversible cause of nephrotic syndrome in HIV infection. N Engl J Med 1991;324:341–2.
[14] Bani-Hani S, Patel V, Larsen CP, Walker PD, Cooke CR, Showkat A. Renal disease in AIDS: it is not always HIVAN. Clin Exp Nephrol 2010;14:263–7.
[15] Wyatt CM, Klotman PE, D'Agati VD. HIV-associated nephropathy: clinical presentation, pathology, and epidemiology in the era of antiretroviral therapy. Semin Nephrol 2008;28:513–22.
[16] Soehardy Z, Hayati SN, Rozita M, Rohana AG, Halim AG, Norella K, et al. Subclinical acquired syphilis masquerading as membranous glomerulonephritis. Med J Malays 2006;61:484–6.
[17] Handoko ML, Duijvestein M, Scheepstra CG, de Fijter CW. Syphilis: a reversible cause of nephrotic syndrome. BMJ Case Rep 2013. http://dx.doi.org/10.1136/bcr-2012-008279.
[18] Ross MJ, Klotman PE. Recent progress in HIV-associated nephropathy. J Am Soc Nephrol 2002;13:2997–3004.
[19] D'Agati V, Appel GB. HIV infection and the kidney. J Am Soc Nephrol 1997;8:138–52.
[20] Ginsberg JM, Chang BS, Matarese RA, Garella S. Use of single voided urine samples to estimate quantitative proteinuria. N Engl J Med 1983;309:1543–6.
[21] McPhee SJ. Secondary syphilis: uncommon manifestations of a common disease. West J Med 1984;140:35–42.
[22] Tatu AL, Popescu M, Rotarescu R, Popescu SD. A rare case of syphilis associated with renal and hepatic involvement. Dermatol Pract Concept 2013;3:53–4.
[23] Tang AL, Thin RN, Croft DN. Nephrotic syndrome and hepatitis in early syphilis. Postgrad Med J 1989;65:14–5.
[24] Hellier MD, Webster AD, Eisinger AJ. Nephrotic syndrome: a complication of secondary syphilis. Br Med J 1971;4:404–5.
[25] Ortega SS, Brito JL, Lémes HL, Socorro CR, Sánchez EM, Martínez JD. Circulating anticoagulant and focal liver lesions associated to rich clinical expression in the secondary syphilis (in Spanish). Med Interna 2007;24:331–4.
[26] Mahto M, Mohammed F, Wilkins E, Mason J, Haboubi NY, Khan AN. Pseudohepatic tumor associated with secondary syphilis in an HIV-positive male. Int J STD AIDS 2006;17:139–41.
[27] Vas W, Thomasson J, Tang-Barton P, Salimi Z, Catral B. Computed tomography and ultrasound appearance of liver disease in secondary syphilis. Diagn Imaging Clin Med 1985;54:1–6.

Original Report: Patient-Oriented, Translational Research

American Journal of Nephrology

Am J Nephrol 2014;39:449–458
DOI: 10.1159/000362567

Received: December 6, 2013
Accepted: March 26, 2014
Published online: May 10, 2014

Carpal Tunnel Surgery as Proxy for Dialysis-Related Amyloidosis: Results from the Japanese Society for Dialysis Therapy

Junichi Hoshino[a] Kunihiro Yamagata[b] Shinichi Nishi[c] Shigeru Nakai[b]
Ikuto Masakane[b] Kunitoshi Iseki[b] Yoshiharu Tsubakihara[b]

[a]Nephrology Center, Toranomon Hospital, and [b]Committee of Renal Data Registry, Japanese Society for Dialysis Therapy, Tokyo, and [c]Division of Nephrology and Kidney Center, Kobe University Graduate School of Medicine, Kobe, Japan

Key Words
Amyloidosis · Carpal tunnel syndrome · Risks · Epidemiology · β_2-microglobulin clearance

Abstract
Background/Aims: This study aims to identify current risk factors for developing dialysis-related amyloidosis using carpal tunnel syndrome (CTS) as proxy for general amyloidosis. ***Methods:*** The cohort consisted of 166,237 patients on dialysis (mean age 66.1 ± 12.4 years; mean dialysis vintage 7.2 ± 6.4 years) who could be followed for a year between 2010 and 2011. Of these, 2,157 (1.30%) needed first-time CTS surgery during the study period. Odds ratios (ORs) for CTS were calculated at a 95% confidence interval (95% CI) after adjusting for age, gender, primary kidney disease, history of smoking, history of hypertension vintage, dialysis modality, use of high-flux membrane, body mass index, serum albumin, Kt/V, normalized protein catabolic rate, C-reactive protein, pretreatment β_2-microglobulin (β_2MG), and β_2MG clearance. ***Results:*** Adjusted ORs of first-time CTS for vintages 10–15, 15–20, 20–25 (referent), 25–30, and >30 years were, respectively, 0.18 (0.12–0.26), 0.43 (0.31–0.62), 1.00, 2.37 (1.64–3.40), and 3.87 (2.52–5.93). Adjusted ORs for ages 40–50, 50–60 (referent), 60–70, 70–80, and >80 were 0.53 (0.30–0.94), 1.00, 1.89 (1.41–2.52), 1.52 (1.08–2.14), and 1.04 (0.60–1.80). Female gender, low serum albumin, and diabetic nephropathy were also associated with CTS. Pretreatment serum β_2MG and β_2MG clearance <80% were not significant, although β_2MG clearance >80% was negatively associated with CTS [OR 0.34 (0.13–0.90)]. ***Conclusion:*** ORs of first-time CTS almost doubled with every 5-year increase in dialysis vintage. ORs of CTS were highest for patients aged 60–70. Other factors associated with CTS were gender, serum albumin, and diabetic nephropathy. β_2MG clearance >80% may decrease the incidence of CTS.

Introduction

Amyloidosis is characterized by deposition of fibrillar amyloid protein in the extracellular space of various organs, resulting in their disorder. Dialysis-related amyloidosis (DRA), one of the systemic amyloidoses, is not uncommon in patients on long-term dialysis treatment. Many clinical manifestations of this process involve deposition of β_2-microglobulin (β_2MG) amyloid in osteoarticular sites [1, 2], especially synovial membranes, resulting in osteoarthropathy of the joints, carpal tunnel syn-

KARGER

0250–8095/14/0395–0449$39.50/0

E-Mail karger@karger.com
www.karger.com/ajn

Kunihiro Yamagata, MD, PhD
Department of Nephrology
Faculty of Medicine, University of Tsukuba
1-1-1, Ten-oudai, Tsukuba, Ibaraki 305-8575 (Japan)
E-Mail k-yamaga@md.tsukuba.ac.jp

drome (CTS), and spondyloarthropathy [3–5]. Serum β_2MG levels among patients with renal failure increased to 20–80 mg/l, 10–50 times higher than the levels found in a healthy population (1.0–2.0 mg/l) [6, 7]. However, it was reported that the serum level of β_2MG – precursor protein of amyloid – may not be the sole cause of DRA [7]: age, dialysis vintage, chronic inflammation, oxidant stress, advanced glycation end product, and some genetic factors have also been considered major risk factors for DRA [8, 9]. Because mean dialysis vintages are getting longer in most medically advanced countries, prevention or management of DRA becomes an increasingly important issue for patients on dialysis.

Since the first case reported by Warren and Otieno [10], CTS is the most common presenting feature of DRA [6]; it results from entrapment of the median nerve in the wrist by deposition of β_2MG amyloid on the transverse carpal ligament or finger flexor synovium [4, 5, 11]. Although an autopsy study showed that prevalence of DRA reached 50% with dialysis vintage 4–7 years [12], clinically, CTS becomes a problem in patients with dialysis vintage over 10 years [8]. It was recently reported that the prevalence of CTS surgery among patients with dialysis vintage 10–15, 15–20, 20–25, and ≥25 years was, respectively, 2.6, 8.8, 23.2, and 51.5%, suggesting a strong association between DRA and dialysis duration [13]. An earlier study reported that the prevalence of CTS started increasing clinically when dialysis vintage was >8 years, and half of the patients on dialysis 20 years were diagnosed with CTS [14].

Methods of dialysis and the quality of dialyzing materials have dramatically improved in the past 40 years, which may have contributed to preventing the onset of DRA or decreasing its incidence. In Japan, hemodialysis filtration (HDF) and biocompatible membranes made with polysulfone (for example) became standard in the 1980s; use of high-flux membranes – which meant lower accumulation of β_2MG than with low-flux dialysis [15] – became widespread in the 1990s. Improvement in the quality of dialysis solution dates to the late 1970s, when acetate-based dialysis solutions were completely replaced by bicarbonate-based dialysis solutions, and reverse osmosis was used to purify water, with endotoxin-cut filters used in the late 1990s.

Since dialysis technology is improving almost year by year, there is a clear need to update data on the incidence proportion (risk) of DRA and the risk factors associated with development of DRA symptoms in the modern era to help guide clinicians in monitoring those at greatest risk of amyloidosis build-up. Ideally, such data should come from a large-scale cohort study, consisting of patients with long dialysis vintages and with few dropouts. Our study analyzed data from recent large-scale nationwide surveys in Japan. It took the first-time onset of CTS surgery as evidence of DRA development in hemodialysis (HD) patients, and identified the risk factors evident in those who had undergone first-time CTS surgery during the study period.

Subjects and Methods

Database Creation

The data were obtained from the nationwide surveys of dialysis patients conducted by the Japanese Society for Dialysis Therapy (JSDT). The creation of these questionnaire-based national surveys has been described previously [16, 17]. Briefly, data of all patients were collected annually from participating facilities. The data covered 297,126 patients dialyzed at 4,152 facilities in the 2010 survey, and 304,592 patients at 4,205 facilities in the 2011 survey. The study cohort consisted of those patients who had received maintenance dialysis for 12 months, December 2010 through December 2011, and whose data could be obtained for both 2010 and 2011. We excluded patients who had been dialyzed fewer than 3 times a week or fewer than 2 h per treatment, those who had received peritoneal dialysis or HD by β_2MG absorption column, those with a history of organ transplantation, and, finally, those with missing variables.

After exclusions, 166,237 patients remained. Demographic data and details of medical history were collected, with information on age, gender, dialysis vintage, height, body weight pre- and postdialysis, presence of diabetes, causes of renal failure, dialysis modality (HD or HDF), use of high-flux membrane, and history of smoking, hypertension, myocardial infarction, cerebrovascular diseases, amputation – *and* CTS surgery. In the cohort, 2,157 patients had received CTS surgery by the end of 2011 with no history of CTS surgery by the end of 2010, and thus had experienced their first CTS surgery during the study period.

Laboratory Measures

Blood samples were drawn and measured at each dialysis center, typically within 24 h, and the most recent laboratory values at the time of survey were collected. Most laboratory values were measured monthly – and at least quarterly – including serum urea nitrogen, creatinine, serum albumin, calcium, phosphate, C-reactive protein (CRP), hemoglobin, intact parathyroid hormone (iPTH) or whole PTH, serum β_2MG, Kt/V, and normalized protein catabolic rate (nPCR) [18]. All these variables were measured before dialysis with the exception of postdialysis serum nitrogen and β_2MG.

Statistical Methods

Data were summarized using proportions and means (±SD) as appropriate. Categorical variables were analyzed with the χ^2 test or Fisher's exact test as appropriate, and continuous variables were compared using the t test, Mann-Whitney U test, Kruskal-Wallis H test, or ANOVA as appropriate. Whole PTH (1–84 PTH) values were converted to iPTH values based on the equation: iPTH = whole PTH × 1.7 [19, 20]. Laboratory values in the data were refined using the following limits: albumin 1.0–5.0 g/dl; height 120–

Table 1. Patients' characteristics among dialysis vintage groups

	Total (n = 166,237)	~5 years (n = 80,135)	~10 years (n = 44,223)	~15 years (n = 22,258)	~20 years (n = 11,117)	~25 years (n = 5,121)	~30 years (n = 2,253)	>30 years (n = 1,130)	p value
Female gender, %	37.4	34.8	38.0	40.7	42.5	44.5	45.3	42.8	<0.01
Modality, %									
Hemodialysis	95.0	97.2	95.1	93.3	90.0	87.5	83.9	78.9	<0.01
HDF	5.0	2.8	4.9	6.7	10.0	12.5	16.1	21.1	
Use of high-flux membrane	84.4	81.7	85.5	87.6	88.8	90.1	90.6	91.6	<0.01
Primary disease, %									
Glomerulonephritis	38.3	25.7	37.1	54.4	69.4	78.9	82.7	84.1	<0.01
Diabetic nephropathy	36.9	47.0	38.9	23.0	10.4	3.5	1.6	1.3	
Nephrosclerosis	8.5	11.1	7.9	5.5	3.5	2.2	1.4	0.6	
ADPKD	3.7	3.1	4.1	4.9	4.5	3.3	1.6	1.7	
Lupus nephritis	0.8	0.7	0.8	1.0	1.2	1.2	0.9	0.8	
Others or unknown	11.8	12.5	11.1	11.2	11.0	11.0	11.8	11.5	
Previous history, %									
Diabetes	42.6	53.6	44.7	27.3	14.0	6.4	4.8	4.4	<0.01
Smoking	15.1	15.3	15.6	14.5	14.2	12.8	12.6	12.5	<0.01
Hypertension	75.2	77.6	77.0	73.2	67.9	63.4	55.8	48.3	<0.01
Myocardial infarction	7.5	7.7	8.1	6.7	6.3	6.2	5.3	5.7	<0.01
Cerebrovascular disease	17.6	16.7	19.9	18.2	16.4	14.7	11.7	10.9	<0.01
Limb amputation	2.6	2.3	3.4	2.9	2.0	1.3	1.6	2.6	<0.01
Age, years	66.1±12.4	67.6±12.7	66.4±12.3	64.2±12.1	62.5±11.4	61.7±10.4	60.7±9.7	61.1±8.0	<0.01
BMI	21.5±3.6	21.9±3.7	21.6±3.6	20.9±3.3	20.4±3.0	20.0±2.9	19.8±2.8	19.5±2.6	<0.01
Urea nitrogen, mg/dl	64.4±15.8	63.3±16.0	64.7±15.6	65.8±15.6	66.2±15.2	66.8±15.5	67.4±15.5	66.4±15.5	<0.01
Creatinine, mg/dl	10.4±2.9	9.6±2.8	11.1±2.7	11.5±2.6	11.5±2.5	11.3±2.4	11.0±2.3	10.3±2.2	<0.01
Calcium, mg/dl	8.9±0.8	8.8±0.8	9.0±0.7	9.2±0.8	9.2±0.8	9.1±0.8	9.1±0.8	9.0±0.9	<0.01
Phosphate, mg/dl	5.3±1.4	5.2±1.4	5.3±1.4	5.3±1.4	5.3±1.4	5.3±1.4	5.3±1.3	5.2±1.3	<0.01
Albumin, g/dl	3.70±0.39	3.67±0.40	3.71±0.37	3.73±0.37	3.74±0.36	3.73±0.37	3.70±0.36	3.65±0.38	<0.01
CRP, mg/dl	0.46±1.41	0.47±1.42	0.47±1.43	0.44±1.37	0.42±1.25	0.45±1.37	0.54±1.72	0.55±1.56	<0.01
Hemoglobin, g/dl	10.6±1.2	10.5±1.2	10.6±1.2	10.6±1.2	10.6±1.2	10.7±1.2	10.6±1.2	10.6±1.3	<0.01
iPTH, pg/ml	152.7±152.2	137.3±127.5	154.6±146.0	181.0±187.1	181.9±199.4	173.7±187.2	174.5±208.5	156.0±192.4	<0.01
β_2MG (pre-dialysis), mg/l	26.9±6.8	24.7±7.0	28.7±6.3	29.2±5.6	28.9±5.4	28.3±5.7	27.3±6.2	24.9±6.4	<0.01
β_2MG (post-dialysis), mg/l	10.9±4.8	11.0±4.9	11.2±5.0	10.8±4.7	10.1±4.1	9.7±4.3	9.3±4.1	8.2±3.1	<0.01
β_2MG clearance, %	65.6±11.3	62.2±11.5	67.0±10.5	69.2±10.1	70.6±9.7	71.5±9.7	71.4±9.7	71.7±9.2	<0.01
Kt/V	1.41±0.30	1.31±0.30	1.45±0.28	1.51±0.28	1.56±0.28	1.58±0.30	1.60±0.31	1.59±0.32	<0.01
Normalized PCR (nPCR)	0.88±0.18	0.84±0.17	0.89±0.17	0.91±0.17	0.93±0.17	0.94±0.18	0.95±0.18	0.93±0.18	<0.01

ADPKD = Autosomal dominant polycystic kidney disease. p values were calculated by χ^2 test or ANOVA as appropriate.

200 cm; body weight 20–150 kg; serum urea nitrogen 10–250 mg/dl; creatinine 3–20 mg/dl; serum calcium 4.0–15.0 mg/dl; phosphate 1.0–15.0 mg/dl; serum albumin 1.0–5.0 g/dl; CRP <50 mg/dl; hemoglobin 5.0– 20.0 g/dl; β_2MG 10.0–100.0 mg/l; Kt/V 0.5–4.0, and nPCR 0.3–2.0. Clearance of β_2MG was calculated by decrease of serum β_2MG values during dialysis, divided by pretreatment β_2MG value. Logistic regression was used to obtain the adjusted odds ratios (ORs) and 95% confidence interval (95% CI) for first-time onset of CTS. In this analysis, the covariates for calculating the adjusted ORs were age, gender, primary kidney disease, history of smoking, history of hypertension, dialysis vintage, dialysis modality, use of high-flux membrane, body mass index (BMI), serum albumin, Kt/V, nPCR, serum creatinine, hemoglobin, CRP, pretreatment β_2MG, and β_2MG clearance at the beginning of observation. Covariates whose p values were <0.2 were included in the multivariate regression analysis. Local polynomial smoothing methods were used to estimate crude risks of CTS among patient groups [21, 22]. All analyses were carried out using Stata® SE Version 12.0 (StataCorp, College Station, Tex., USA).

Results

After exclusions according to the criteria cited above, 166,237 patients were analyzed (female 37%; mean age 66.1 ± 12.4 years; mean dialysis vintage 7.2 ± 6.4 years). Background characteristics of these patients are shown in table 1. As dialysis vintage increased, the proportion of females, of glomerulonephritis as a primary disease, and of patients on HDF all increased, as did mean β_2MG clearance and mean Kt/V. On the other hand, the proportion of diabetic nephropathy and nephrosclerosis as primary diseases, previous history of diabetes, smoking, hypertension, cardiovascular disease, mean age, mean BMI, and mean albumin all decreased as dialysis vintage increased. In this cohort, 2,157 patients (1.30%) had surgery during the December 2010–December 2011 study period

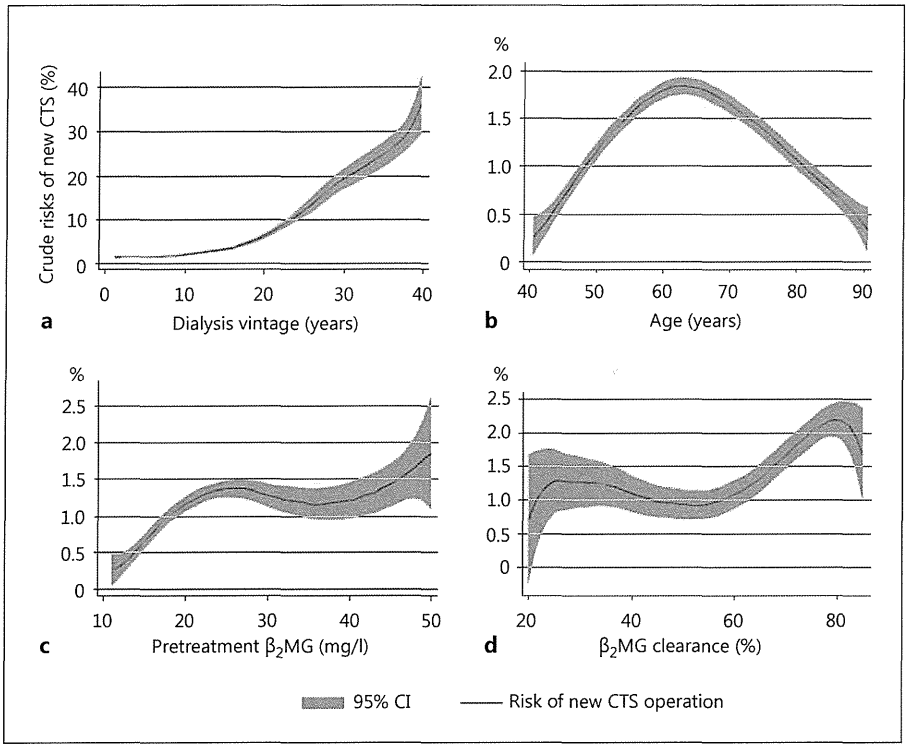

Fig. 1. Crude risks and 95% CI of CTS by dialysis vintage (**a**), age (**b**), pretreatment serum β_2MG (**c**), and β_2MG clearance during dialysis (**d**).

for a first-time onset of CTS. The proportion of females was higher in this CTS group than in the remainder of the cohort with no CTS (50.4 vs. 37.3%, $p < 0.01$); the proportion was also higher in the CTS group of those receiving HDF (13.7 vs. 4.9%, $p < 0.01$) and use of high-flux membrane (88.5 vs. 84.4%, $p < 0.01$) and so was glomerulonephritis as the primary kidney disease (62.0 vs. 38.0%, $p < 0.01$). However, the CTS group had lower BMI (20.7 ± 3.3 vs. 21.5 ± 3.6, $p < 0.01$), serum albumin (3.66 ± 0.36 vs. 3.70 ± 0.39 g/dl, $p < 0.01$), and β_2MG after dialysis (10.2 ± 4.6 vs. 10.9 ± 4.8 mg/l, $p < 0.01$), but had higher β_2MG before dialysis (27.3 ± 6.5 vs. 26.9 ± 6.8 mg/l, $p < 0.01$), higher serum urea nitrogen (65.8 ± 15.7 vs. 64.4 ± 15.8 mg/dl, $p < 0.01$), CRP (0.54 ± 1.61 vs. 0.46 ± 1.40 mg/dl, $p = 0.02$), and Kt/V (1.53 ± 0.30 vs. 1.40 ± 0.30, $p < 0.01$). Mean dialysis vintage in the CTS group was 18.2 ± 9.5 years, but only 7.0 ± 6.2 years for those with no CTS ($p < 0.01$) (online suppl. table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000362567).

Distribution of the (Unadjusted) Incidence Proportion of CTS Onset

Next, we examined the crude incidence proportion (risk) of CTS surgery for dialysis vintage, age, serum β_2MG before dialysis, and β_2MG clearance. As shown in figure 1, online supplementary figure 1, and online supplementary table 2, the crude risk doubled with every 5-year vintage increase from 10–15, 15–20, 20–25, 25–30, and >30 years, the percent of risk being, respectively, 1.5 (1.3–1.6), 3.4 (3.0–3.7), 7.8 (7.1–8.6), 14.3 (12.9–15.7), and 21.3 (18.9–23.7). Note that the crude risk significantly increased in patients with dialysis vintage ≥25 years. On the other hand, the shape of the graph was different when we categorized by age. The crude risk was highest at age 64 (1.9%, 95% CI 1.6–2.3) and in the 60–70 age group (1.8%, 95% CI 1.7–1.9), but was lower as patients were younger or older than that range. When we categorized by pretreatment β_2MG levels, the percent of crude risk of CTS surgery when pretreatment β_2MG was <20, 20–25, 25–30, 30–35, 35–40, 40–45, and >45 mg/l was, respectively, 0.9 (0.7–1.0), 1.5 (1.4–1.7), 1.3 (1.2–1.4), 1.3 (1.2–1.4), 1.2 (0.9–1.4), 1.4 (1.0–1.8), and 1.5 (1.0–2.1). So the crude risk diminished with pretreatment β_2MG from 20 to 40 mg/l, then increased as pretreatment β_2MG increased past 40 mg/l. Surprisingly, when we categorized by β_2MG clearance, the crude risk of CTS surgery gradually increased up to 80% β_2MG clearance. That risk was 1.3% (1.1–1.5) with β_2MG clearance 60–70%, increasing to 2.0% (1.7–2.3) with 70–80% β_2MG clearance, but with β_2MG clearance >80% the risk decreased to 1.1% (0.4–1.9).

Distribution of the Adjusted ORs for CTS Surgery

We then analyzed adjusted ORs for CTS surgery. As shown in figure 2 and table 2, the adjusted ORs for the various dialysis vintage ranges, with 20–25 years as referent, were 0.03 (95% CI 0.02–0.05) for vintage <5 years, 0.07 (0.05–0.10) for vintage 5–10 years, 0.18 (0.12–0.26) for vintage 10–15 years, 0.43 (0.31–0.62) for vintage 15–20 years, 2.37 (1.64–3.40) for vintage 25–30 years, and 3.87 (2.52–5.93) >30 years – after adjusting for patients' other background factors (age, gender, primary kidney disease, history of smoking, history of hypertension, dialysis modality, use of high-flux membrane, and BMI) and laboratory values (albumin, Kt/V, nPCR, CRP, and β_2MG clearance) (model 2). These adjusted ORs were very similar if we include pretreatment β_2MG in the analysis. The upward trend of adjusted ORs was similar to that of the unadjusted ORs, suggesting a very strong linear relationship with CTS surgery (online suppl. fig. 2; online suppl. table 2).

When categorizing the study group by age, the adjusted ORs for CTS surgery, with age 50–60 as referent, were 0.18 (0.02–1.27) for age ≤40, 0.53 (0.30–0.94) for age 40–50, 1.89 (1.41–2.52) for age 60–70, 1.52 (1.08–2.14) for age 70–80, and 1.04 (0.60–1.80) for age >80 – after adjusting for all the other factors cited above (fig. 2; online suppl. table 2). The unadjusted ORs for CTS surgery for the same age ranges were, respectively, 0.18 (0.10–0.30), 0.48 (0.39–0.60), 1.00 (referent), 1.18 (1.05–1.32), 0.78 (0.68–0.88), and 0.47 (0.39–0.56). In both unadjusted and adjusted models, the OR was highest in the age group 60–70 (table 2; online suppl. fig. 2).

In contradistinction to the two covariates above, neither pretreatment serum β_2MG values nor β_2MG clearance, in general, showed particular trends in relationship with the adjusted ORs for CTS surgery, although one aspect of β_2MG clearance did bear such a relationship. With pretreatment serum β_2MG <20 mg/l as referent, the adjusted ORs for CTS surgery were: for β_2MG 20–25 mg/l, 1.10 (0.88–1.39); 0.79 (0.63–1.00) for 25–30 mg/l; 0.83 (0.65–1.07) for 30–35 mg/l; 0.82 (0.60–1.12) for 35–40 mg/l; 0.86 (0.59–1.25) for β_2MG >40 mg/l – after adjust-

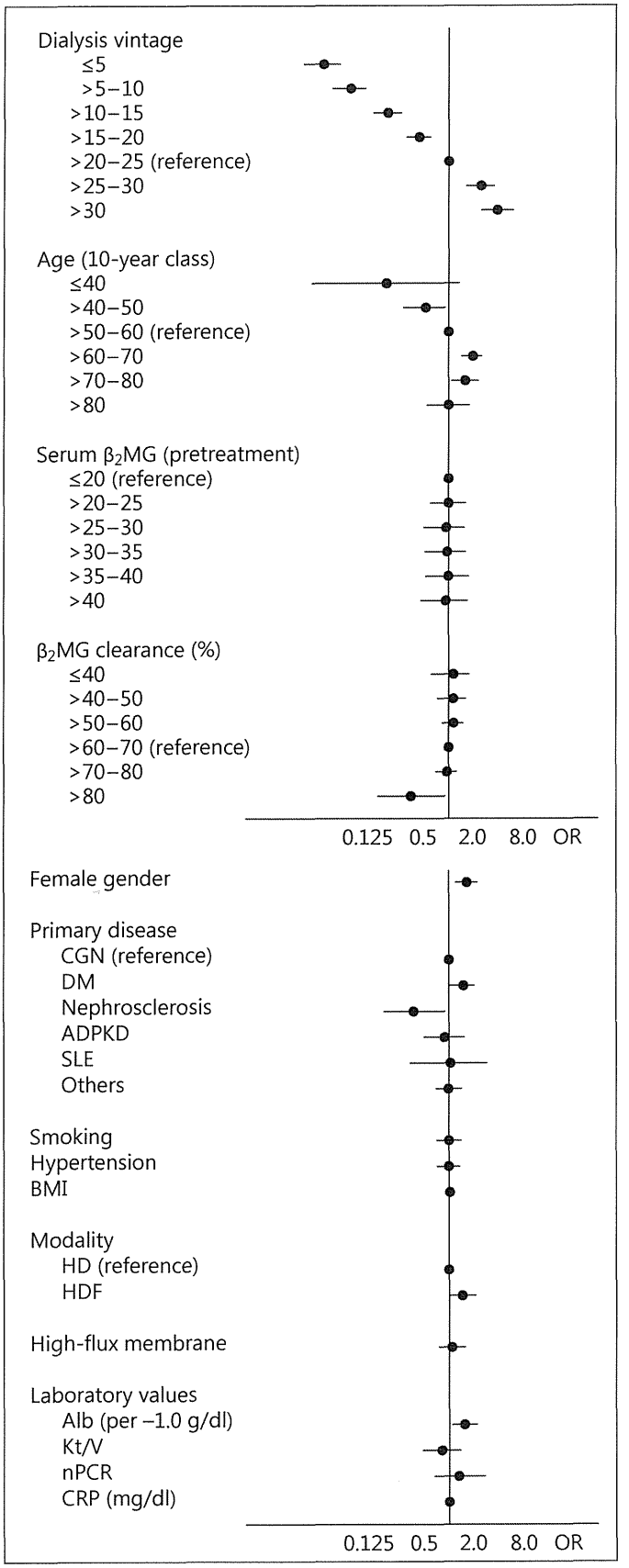

Fig. 2. Adjusted ORs of CTS. These ORs were adjusted by dialysis vintage, age, gender, primary kidney disease, history of smoking, history of hypertension, dialysis modality, use of high-flux membrane, BMI, serum albumin (Alb), Kt/V, nPCR, and serum β_2MG clearance. CGN = Chronic glomerulonephritis; DM = diabetic nephropathy; ADPKD = autosomal dominant polycystic kidney disease; SLE = systemic lupus erythematosus.

Table 2. ORs for first-time CTS

	Model 1			Model 2			Model 3		
	OR	95% CI	p value	OR	95% CI	p value	OR	95% CI	p value
Dialysis vintage									
≤5	0.02	0.02–0.03	0.00	0.03	0.02–0.05	0.00	0.03	0.02–0.05	0.00
≤10	0.05	0.04–0.07	0.00	0.07	0.05–0.10	0.00	0.07	0.05–0.10	0.00
≤15	0.16	0.13–0.20	0.00	0.18	0.12–0.26	0.00	0.18	0.12–0.26	0.00
≤20	0.41	0.34–0.50	0.00	0.43	0.31–0.62	0.00	0.44	0.31–0.62	0.00
≤25	1.00			1.00			1.00		
≤30	1.94	1.57–2.38	0.00	2.37	1.64–3.40	0.00	2.32	1.61–3.35	0.00
>30	3.00	2.35–3.82	0.00	3.87	2.52–5.93	0.00	3.75	2.43–5.80	0.00
Age group									
≤40	0.29	0.13–0.61	0.00	0.18	0.02–1.27	0.09	0.17	0.02–1.27	0.09
≤50	0.46	0.34–0.63	0.00	0.53	0.30–0.94	0.03	0.53	0.30–0.94	0.03
≤60	1.00			1.00			1.00		
≤70	1.56	1.33–1.83	0.00	1.89	1.41–2.52	0.00	1.87	1.40–2.50	0.00
≤80	1.55	1.29–1.86	0.00	1.52	1.08–2.14	0.02	1.51	1.07–2.12	0.02
>80	1.37	1.04–1.81	0.02	1.04	0.60–1.80	0.89	1.03	0.59–1.79	0.91
Female gender	1.54	1.36–1.75	0.00	1.63	1.30–2.06	0.00	1.63	1.29–2.05	0.00
Primary disease									
CGN	1.00			1.00			1.00		
DM	1.41	1.19–1.67	0.00	1.52	1.12–2.06	0.01	1.53	1.13–2.07	0.01
Nephrosclerosis	0.81	0.58–1.12	0.20	0.39	0.17–0.90	0.03	0.39	0.17–0.90	0.03
ADPKD	0.90	0.65–1.24	0.52	0.88	0.50–1.55	0.66	0.88	0.50–1.54	0.66
SLE	1.27	0.71–2.26	0.41	0.99	0.35–2.80	0.99	1.00	0.35–2.81	0.99
Others	0.92	0.75–1.12	0.39	1.03	0.72–1.48	0.89	1.03	0.72–1.49	0.86
Smoking	1.24	1.04–1.47	0.02	1.02	0.73–1.43	0.90	1.02	0.73–1.44	0.89
Hypertension	1.00	0.88–1.14	0.98	0.95	0.76–1.18	0.63	0.95	0.76–1.18	0.63
BMI	1.03	1.01–1.05	0.00	1.02	0.99–1.05	0.28	1.02	0.99–1.05	0.27
Modality									
HD	1.00			1.00			1.00		
HDF	1.31	1.11–1.56	0.00	1.43	1.03–1.97	0.03	1.42	1.03–1.97	0.03
High-flux membrane	0.95	0.80–1.14	0.60	1.11	0.76–1.60	0.59	1.11	0.76–1.61	0.59
Laboratory values									
Alb (per –1 g/dl)	1.15	0.96–1.36	0.12	1.49	1.10–2.02	0.01	1.49	1.10–2.02	0.01
Kt/V	0.82	0.65–1.03	0.09	0.80	0.50–1.28	0.36	0.81	0.50–1.29	0.37
nPCR	1.17	0.82–1.66	0.40	1.37	0.72–2.62	0.34	1.40	0.73–2.66	0.31
CRP (mg/dl)	1.01	0.97–1.05	0.52	1.01	0.94–1.08	0.84	1.01	0.94–1.08	0.83
Serum β_2MG, pretreatment									
≤20	1.00						1.00		
≤25	1.10	0.88–1.39	0.40				1.01	0.65–1.57	0.98
≤30	0.79	0.63–1.00	0.05				0.84	0.54–1.30	0.43
≤35	0.83	0.65–1.07	0.15				0.92	0.57–1.46	0.71
≤40	0.82	0.60–1.12	0.22				1.00	0.57–1.74	0.99
>40	0.86	0.59–1.25	0.43				0.86	0.43–1.70	0.66
β_2MG clearance									
≤40				1.16	0.68–1.97	0.59	1.16	0.68–1.97	0.60
≤50				1.16	0.75–1.81	0.51	1.16	0.74–1.80	0.52
≤60				1.15	0.85–1.55	0.36	1.15	0.85–1.55	0.37
≤70				1.00			1.00		
≤80				0.95	0.72–1.26	0.71	0.95	0.72–1.26	0.72
>80				0.34	0.13–0.90	0.03	0.35	0.13–0.91	0.03

CGN = Chronic glomerulonephritis; DM = diabetic nephropathy; ADPKD = autosomal dominant polycystic kidney disease; SLE = systemic lupus erythematosus.

Model 1 includes patient background (age, gender, primary kidney disease, history of smoking, history of hypertension, dialysis vintage, dialysis modality, BMI), serum albumin, Kt/V, nPCR, and pretreatment β_2MG at the beginning of observation.

Model 2 includes patient background (age, gender, primary kidney disease, history of smoking, history of hypertension, dialysis vintage, dialysis modality, BMI), serum albumin, Kt/V, nPCR, and β_2MG clearance at the beginning of observation.

Model 3 includes model 2 plus pretreatment β_2MG.

ing for the same factors as above (fig. 2; table 2; online suppl. fig. 2). Since there was no significant difference between the various levels of pretreatment β_2MG values, those values were evidently not associated with need for CTS surgery. On the other hand, looking at levels of β_2MG clearance, with 60–70% as reference, the ORs for CTS surgery were, for β_2MG clearance <40%, 1.16 (0.68–1.97), 1.16 (0.75–1.81) at 40–50%, 1.15 (0.85–1.55) at 50–60%, but 0.95 (0.72–1.26) at 70–80%, and 0.34 (0.13–0.90) for β_2MG clearance >80% – again after adjusting for the same factors (fig. 2; table 2; online suppl. fig. 2). These findings suggest that β_2MG clearance >80% may be associated with decreasing risk of CTS surgery.

However, our analysis also identified factors that were positively associated with need for CTS surgery: being female [OR 1.63 (1.30–2.06)], having lower serum albumin [OR 1.49 (1.10–2.02) per 1 g/dl decrease], having diabetic nephropathy rather than chronic glomerulonephritis as the primary kidney disease [OR 1.52 (1.12–2.06)], and having HDF rather than HD [OR 1.43 (1.03–1.97)] (fig. 2; table 2).

Discussion

There are currently more than 290,000 Japanese chronic HD patients, with 6.4% of them having been on HD for over 20 years [23]. Because appearance of DRA is closely related to dialysis vintage, our large-scale cohort, consisting of patients with relatively longer dialysis vintage, seemed the ideal population to clarify the risk and factors associated with DRA.

CTS is one of the major complications of DRA, and is recognized as perhaps the most evident marker of DRA development in a patient. Since the build-up of amyloidosis is otherwise difficult to detect – especially in such a large population as in our cohort – the fact of CTS surgery stands as a handy and reliable proxy for that build-up. To minimize the possibility that the CTS surgery was for recrudescence of a previous condition, only those patients who had had the surgery for the first time during the December 2010–December 2011 study period, with no history of CTS before December 2010, were included in the group analyzed for the risk factors associated with the development of CTS.

There are many new findings in this analysis. First, in our dialysis population, the annual risk of *new* CTS surgery (as noted, those with a history of CTS surgery were excluded from our study group) was much higher (1.3%) than in the general population (0.11%) [24]. Second, the risk of CTS almost doubled with each 5-year increase in dialysis vintage. Although it has been reported that the prevalence of patients who needed new CTS surgery was closely associated with dialysis vintage [13], ours is the first report showing the risk of first-time CTS surgery by dialysis vintage – and showing it in a large dialysis cohort. Third, the relationship between risk of CTS surgery and age was not linear but mountain-shaped, peaking at around age 64. Fourth, neither pretreatment serum β_2MG level nor β_2MG clearance (up to 80%) was associated with risk of CTS surgery. However, our findings imply that it might be possible to decrease the risk of onset of DRA by achieving β_2MG clearance >80%. Finally, our study showed that risk factors associated with need for CTS surgery, which is considered a good proxy for the development of DRA [6, 25], may nowadays differ slightly from the factors previously reported. Here, the risk factors were dialysis vintage, age (up to 70 years), primary kidney disease (diabetic or not), lower serum albumin, modality, and β_2MG clearance (<80%). Surprisingly, CRP – as a proxy for chronic inflammation – was not a significant risk factor in this analysis.

It was previously reported that age, dialysis vintage, lower serum albumin, chronic inflammation, oxidant stress, advanced glycation end product, and some genetic factors were the main risk factors for DRA [8, 9, 26]. Although age, dialysis vintage, and genetic factors are naturally unchangeable, many attempts have been made to reduce chronic inflammation and oxidant stress, which have been considered treatable risk factors. It was reported that dialysis with a polysulfone membrane, rather than with cuprofan, may decrease the risk of new DRA since polysulfone is biocompatible [6, 27, 28]. Moreover, use of lower contamination of endotoxin dialysis solutions was reported to decrease the incidence of onset of CTS [29]. Nowadays, >97% of JSDT dialysis facilities could meet the JSDT standard for bacterial cell counts in dialysis fluid (<100 CFU/ml) [30], and most of the facilities use biocompatible membranes. These improvements in dialysis technology – including bicarbonate-based dialysis solutions, high-flux and biocompatible membranes, reverse osmosis machines and endotoxin-cut filters – all calculated to lower the incidence of CTS, may have changed the risk factors for development of DRA. And, in fact, based on the JSDT annual report, among patients with dialysis vintage 20–25 years, the prevalence of CTS surgery decreased from 48.0% in the 1999 survey to 23.2% in the 2010 survey, and from 70.8 to 51.5% among patients with dialysis vintage ≥25 years [13]. In this analysis, the use of high-flux membrane was not associated with new

CTS surgery, which might be because of the short period of observation and the clinical tendency to choose high-flux membrane for patients with high risk of CTS.

The effect on DRA of an elevated concentration of circulating β_2MG is still a matter of controversy. Although marked elevation of serum β_2MG is a prerequisite for the formation of β_2MG fibrils [1, 31, 32], the K/DOQI clinical guideline suggests that, on the other hand, the level of serum β_2MG may not be directly associated with onset of DRA symptoms [33]. Our adjusted analysis suggests that in the patients we studied, high pretreatment β_2MG level was not associated with DRA, though the crude risk of DRA was higher in these patients. The difference in findings between the unadjusted and adjusted analyses suggests that many other factors confound the association between pretreatment β_2MG level and DRA, suggesting, in turn, that pretreatment β_2MG cannot be a treatment target for reduction of new DRA, which is consistent with the K/DOQI clinical guideline. Rather, it is reported that β_2MG level could be a possible marker for mortality in dialysis patients [34].

In our study, having diabetic nephropathy rather than chronic glomerulonephritis increased the odds of CTS surgery [OR 1.50 (1.11–2.04)]. Previously, it was reported that the prevalence of CTS surgery in Japan's dialysis population was lower among diabetic patients (1.2%) than among the rest of that population (4.3%) [13]. This discrepancy could be explained by the shorter dialysis vintage among diabetic patients. In fact, in the non-dialysis population, it was reported that prevalence of CTS was higher among diabetics (8.6%) [35] than in the general population (2.7%) [36]. This is consistent with the fact that formation of advanced glycation end product is associated with development of diabetic nephropathy. Furthermore, there has been an unfortunate shift in the percentages of primary kidney diseases requiring dialysis. For example, the proportion of chronic glomerulonephritis decreased from 61.7% in the 1991 Japanese survey to 36.2% in the 2010 survey while the proportion of diabetic nephropathy increased from 16.4 to 35.9% in those respective surveys [13]. So, despite the improvement in dialysis technology, the increasing prevalence of diabetic nephropathy may increase the risk of DRA in the future.

In this analysis, surprisingly, age is not linearly associated with need for CTS surgery. The upward slope peaked at around 64 years, then declined in both unadjusted and adjusted models. The exact reason for this is unclear, but it may be that generation of amyloid fibrils, amyloid P component, and/or glycosaminoglycans/proteoglycans may decrease in the older population, and all these factors are necessary for growth of amyloid fibrils [3]. In addition, the number excluded from surgery due to poor general condition may be higher among older patients. A different proxy marker for DRA might be needed to clarify a more exact risk of DRA in the elderly.

The therapeutic effectiveness of HDF for DRA is still controversial. In some studies, dialysis by HDF and hemofiltration, which may contribute to the decrease of serum β_2MG levels – or dialysis by β_2MG absorption column – were reported to possibly decrease the incidence of DRA or its progression [6, 37, 38]. However, the patients in our study who received HDF had higher ORs for CTS surgery than did those receiving standard HD. That is not to suggest that HDF is likely to cause CTS: ours was an observational study. As shown in table 1, the proportion of HDF was markedly higher in patients with longer dialysis vintages. For example, the proportion of HDF in patients with dialysis vintages of less than 5 years, of 15–20 years, and more than 30 years was, respectively, 2.8, 10.0, and 21.2%. Furthermore, the average dialysis vintage of HDF patients was significantly longer than that of HD patients (11.2 ± 8.1 vs. 7.0 ± 6.2 years, $p < 0.001$) (online suppl. table 3). Therefore, risk of CTS surgery may be greater in HDF patients than HD patients because of their longer dialysis vintage and the higher proportion of females among them, both of which are strong risk factors for DRA. With regard to β_2MG clearance, the average β_2MG clearance in HDF patients was significantly higher than that in HD patients (69.8 ± 10.9 and 59.8 ± 12.2%, respectively, $p < 0.001$). However, this difference did not decrease the risk of CTS surgery in our HDF population, probably because they had other risks of CTS – such as dialysis vintage – or because their β_2MG clearance did not exceed 80%. Prospective and long longitudinal studies are needed to clarify any possible effect of HDF on DRA.

There were some limitations in our study. First, the observation period was relatively short. There could be other risk factors associated with DRA that chronically affect the development of DRA, e.g. chronic inflammation. Second, since we used CTS surgery as a proxy for symptomatic DRA, it could be possible that our outcome did not reflect the exact appearance of symptomatic CTS. In other words, some patients may already have had other DRA symptoms before the onset of CTS, such as trigger finger, spinal canal stenosis, and destructive spondyloarthritis, although CTS is considered one of the best markers for detecting onset of DRA [6]. In addition, poor systemic condition, especially in elder patients, may limit the eligibility for CTS surgery. Third, dialysis conditions in Japan differ from those in the USA and Europe. For ex-

ample, the mean dialysis vintage in Japan (8.9 years) was reported as much longer than in the USA (4.1 years) and in the UK (4.5 years), and mean ages were somewhat longer: 65.5 in Japan versus 62.6 in the USA, and 62.6 in the UK [39]. These differences in patient groups may impact the different incidence of CTS in Japan and other countries. In addition, the ratio of vascular catheter use, associated with a high incidence of inflammation, was much lower in Japan (2%) than in the USA (25%) and in European countries (10% in Germany, 12% in Italy, and 21% in Spain) [40]. This may also be associated with the difference in incidence of CTS between Japan and other countries. Finally, our database derived from annual surveys, which made it impossible to detect the onset of need for a CTS operation by month. Despite these limitations, we believe that these analyses identify the more reliable risks and factors associated with symptomatic DRA, since our database was one of the largest ever used to study dialysis patients, and it included longer dialysis vintages, allowing more reliable estimation and minimization of individual variations or selection bias.

In conclusion, to help guide clinicians in monitoring those most at risk, this study demonstrates several risk factors for DRA in this modern era – as evidenced by need for first-time CTS. First, dialysis vintage was a very strong risk factor for developing DRA. Second, the patients' age was also an important factor for developing DRA – although, interestingly, the risk of developing DRA decreased after about age 64. Third, pretreatment serum β_2MG value was not associated with development of DRA. Finally, however, contrary to the insignificance of pretreatment β_2MG levels, β_2MG clearance of more than 80% seemed to be a factor in preventing development of DRA.

Acknowledgements

We thank the members of the subcommittee of local cooperation for all their efforts, the staff members of dialysis facilities who participated in the survey and responded to the questionnaires, and Dr. Chiho Iseki for data management.

This study was supported by J.H.'s research grants from the Okinaka Memorial Institute for Medical Research, in part by a Grant-in-Aid for Amyloidosis Research, Research on Intractable Disease, from the Ministry of Health, Labor, and Welfare of Japan (H23-intractable-general-012), and in part by K.Y.'s Grant-in-Aid for Research on Establishment of Consulting System to Avoid Progression of CKD, Research on Renal Diseases, from the Ministry of Health, Labor and Welfare of Japan (H24-intractable-kidney-007).

Disclosure Statement

The authors have no conflicts of interest to disclose.

References

1 Gejyo F, Yamada T, Odani S, Nakagawa Y, Arakawa M, Kunitomo T, Kataoka H, Suzuki M, Hirasawa Y, Shirahama T, et al: A new form of amyloid protein associated with chronic hemodialysis was identified as β_2-microglobulin. Biochem Biophys Res Commun 1985;129:701–706.

2 Gejyo F, Odani S, Yamada T, Honma N, Saito H, Suzuki Y, Nakagawa Y, Kobayashi H, Maruyama Y, Hirasawa Y, et al: Beta-2-microglobulin: a new form of amyloid protein associated with chronic hemodialysis. Kidney Int 1986;30:385–390.

3 Ypersele C, Drueke T: Dialysis Amyloid. New York, Oxford University Press, 1996.

4 Okuda I, Ubara Y, Takaichi K, Kitajima I, Motoi N, Hara S, Kokubo T: Genital β_2-microglobulin amyloidoma in a long-term dialysis patient. Am J Kidney Dis 2006;48:e35–e39.

5 Cobby MJ, Adler RS, Swartz R, Martel W: Dialysis-related amyloid arthropathy: MR findings in four patients. AJR Am J Roentgenol 1991;157:1023–1027.

6 Gejyo F, Suzuki S, Arakawa M: Dialysis-related amyloidosis and clinical significance of extracorporeal removal of β_2-microglobulin. Ther Apher 1997;1:126–128.

7 Yamamoto S, Kazama JJ, Narita I, Naiki H, Gejyo F: Recent progress in understanding dialysis-related amyloidosis. Bone 2009; 45(suppl 1):S39–S42.

8 Gejyo F, Narita I: Current clinical and pathogenetic understanding of β_2-M amyloidosis in long-term haemodialysis patients. Nephrology (Carlton) 2003;8(suppl):S45–S49.

9 Cruz DN, de Cal M, Ronco C: Oxidative stress and anemia in chronic hemodialysis: the promise of bioreactive membranes. Contrib Nephrol. Basel, Karger, 2008, vol 161, pp 89–98.

10 Warren DJ, Otieno LS: Carpal tunnel syndrome in patients on intermittent haemodialysis. Postgrad Med J 1975;51:450–452.

11 Kiss E, Keusch G, Zanetti M, Jung T, Schwarz A, Schocke M, Jaschke W, Czermak BV: Dialysis-related amyloidosis revisited. AJR Am J Roentgenol 2005;185:1460–1467.

12 Jadoul M, Garbar C, Noel H, Sennesael J, Vanholder R, Bernaert P, Rorive G, Hanique G, van Ypersele de Strihou C: Histological prevalence of β_2-microglobulin amyloidosis in hemodialysis: a prospective post-mortem study. Kidney Int 1997;51:1928–1932.

13 Nakai S, Iseki K, Itami N, Ogata S, Kazama JJ, Kimata N, Shigematsu T, Shinoda T, Shoji T, Suzuki K, Taniguchi M, Tsuchida K, Nakamoto H, Nishi H, Hashimoto S, Hasegawa T, Hanafusa N, Hamano T, Fujii N, Masakane I, Marubayashi S, Morita O, Yamagata K, Wakai K, Wada A, Watanabe Y, Tsubakihara Y: An overview of regular dialysis treatment in Japan (as of 31 December 2010). Ther Apher Dial 2012;16:483–521.

14 Shimada H, Suzuki M: Dialysis-related amyloidosis: clinical guideline for nephrology and urology, 2006 (in Japanese). Jin to Touseki 2006;61:573–577.

15 Locatelli F, Martin-Malo A, Hannedouche T, Loureiro A, Papadimitriou M, Wizemann V, Jacobson SH, Czekalski S, Ronco C, Vanholder R: Effect of membrane permeability on survival of hemodialysis patients. J Am Soc Nephrol 2009;20:645–654.
16 Shoji T, Masakane I, Watanabe Y, Iseki K, Tsubakihara Y: Elevated non-high-density lipoprotein cholesterol (non-HDL-C) predicts atherosclerotic cardiovascular events in hemodialysis patients. Clin J Am Soc Nephrol 2011;6:1112–1120.
17 Iseki K, Nakai S, Yamagata K, Tsubakihara Y: Tachycardia as a predictor of poor survival in chronic haemodialysis patients. Nephrol Dial Transplant 2011;26:963–969.
18 Shinzato T, Nakai S, Fujita Y, Takai I, Morita H, Nakane K, Maeda K: Determination of Kt/V and protein catabolic rate using pre- and postdialysis blood urea nitrogen concentrations. Nephron 1994;67:280–290.
19 Gao P, Scheibel S, D'Amour P, John MR, Rao SD, Schmidt-Gayk H, Cantor TL: Development of a novel immunoradiometric assay exclusively for biologically active whole parathyroid hormone 1–84: implications for improvement of accurate assessment of parathyroid function. J Bone Miner Res 2001; 16:605–614.
20 Guideline Working Group, Japanese Society for Dialysis Therapy: Clinical practice guideline for the management of secondary hyperparathyroidism in chronic dialysis patients. Ther Apher Dial 2008;12:514–525.
21 Delaigle A, Fan J, Carroll RJ: A design-adaptive local polynomial estimator for the errors-in-variables problem. J Am Statist Assoc 2009; 104:348–359.
22 Gutierrez RG, Linhart JM, Pitblado JS: From the help desk: local polynomial regression and Stata plugins. Stata J 2003;3:412–419.
23 Nakai S, Iseki K, Itami N, Ogata S, Kazama JJ, Kimata N, Shigematsu T, Shinoda T, Shoji T, Suzuki K, Taniguchi M, Tsuchida K, Nakamoto H, Nishi H, Hashimoto S, Hasegawa T, Hanafusa N, Hamano T, Fujii N, Masakane I, Marubayashi S, Morita O, Yamagata K, Wakai K, Wada A, Watanabe Y, Tsubakihara Y: Overview of regular dialysis treatment in Japan (as of 31 December 2009). Ther Apher Dial 2012;16:11–53.
24 Gelfman R, Melton LJ 3rd, Yawn BP, Wollan PC, Amadio PC, Stevens JC: Long-term trends in carpal tunnel syndrome. Neurology 2009;72:33–41.
25 Schwarz A, Keller F, Seyfert S, Poll W, Molzahn M, Distler A: Carpal tunnel syndrome: a major complication in long-term hemodialysis patients. Clin Nephrol 1984;22:133–137.
26 Busch M, Schwenzky A, Franke S, Stein G, Wolf G: Advanced glycation end products and β_2-microglobulin as predictors of carpal tunnel syndrome in hemodialysis patients. Blood Purif 2012;34:3–9.
27 Kuchle C, Fricke H, Held E, Schiffl H: High-flux hemodialysis postpones clinical manifestation of dialysis-related amyloidosis. Am J Nephrol 1996;16:484–488.
28 Locatelli F, Hannedouche T, Jacobson S, La Greca G, Loureiro A, Martin-Malo A, Papadimitriou M, Vanholder R: The effect of membrane permeability on ESRD: design of a prospective randomised multicentre trial. J Nephrol 1999;12:85–88.
29 Baz M, Durand C, Ragon A, Jaber K, Andrieu D, Merzouk T, Purgus R, Olmer M, Reynier JP, Berland Y: Using ultrapure water in hemodialysis delays carpal tunnel syndrome. Int J Artif Organs 1991;14:681–685.
30 Masakane I, Takemoto Y, Nakai S, Tsubakihara Y, Akiba T, Watanabe Y, Iseki K: Bacteriological water quality in the central dialysis fluid delivery system from the survey of the Japanese Society for Dialysis Therapy. Blood Purif 2009;27(suppl 1):11–16.
31 Liabeuf S, Lenglet A, Desjardins L, Neirynck N, Glorieux G, Lemke HD, Vanholder R, Diouf M, Choukroun G, Massy ZA: Plasma β_2-microglobulin is associated with cardiovascular disease in uremic patients. Kidney Int 2012;82:1297–1303.
32 Drueke TB, Massy ZA: Beta-2-microglobulin. Semin Dial 2009;22:378–380.
33 K/DOQI clinical practice guidelines for bone metabolism and disease in chronic kidney disease. Am J Kidney Dis 2003;42:S1–S201.
34 Cheung AK, Rocco MV, Yan G, Leypoldt JK, Levin NW, Greene T, Agodoa L, Bailey J, Beck GJ, Clark W, Levey AS, Ornt DB, Schulman G, Schwab S, Teehan B, Eknoyan G: Serum β_2-microglobulin levels predict mortality in dialysis patients: results of the HEMO study. J Am Soc Nephrol 2006;17:546–555.
35 Kiani J, Goharifar H, Moghimbeigi A, Azizkhani H: Prevalence and risk factors of five most common upper extremity disorders in diabetics. J Res Health Sci 2014;14:92–95.
36 Atroshi I, Gummesson C, Johnsson R, Ornstein E, Ranstam J, Rosen I: Prevalence of carpal tunnel syndrome in a general population. JAMA 1999;282:153–158.
37 Locatelli F, Mastrangelo F, Redaelli B, Ronco C, Marcelli D, La Greca G, Orlandini G: Effects of different membranes and dialysis technologies on patient treatment tolerance and nutritional parameters. The Italian Cooperative Dialysis Study Group. Kidney Int 1996;50:1293–1302.
38 Gejyo F, Kawaguchi Y, Hara S, Nakazawa R, Azuma N, Ogawa H, Koda Y, Suzuki M, Kaneda H, Kishimoto H, Oda M, Ei K, Miyazaki R, Maruyama H, Arakawa M, Hara M: Arresting dialysis-related amyloidosis: a prospective multicenter controlled trial of direct hemoperfusion with a β_2-microglobulin adsorption column. Artif Organs 2004;28:371–380.
39 2012 Annual Report of the Dialysis Outcomes and Practice Patterns Study: Hemodialysis data 1997–2011. Ann Arbor, Arbor Research Collaborative for Health, 2012.
40 Ethier J, Mendelssohn DC, Elder SJ, Hasegawa T, Akizawa T, Akiba T, Canaud BJ, Pisoni RL: Vascular access use and outcomes: an international perspective from the Dialysis Outcomes and Practice Patterns Study. Nephrol Dial Transplant 2008;23:3219–3226.

BASIC RESEARCH www.jasn.org

Overexpression of Mafb in Podocytes Protects against Diabetic Nephropathy

Naoki Morito,* Keigyou Yoh,* Masami Ojima,† Midori Okamura,† Megumi Nakamura,† Michito Hamada,† Homare Shimohata,‡ Takashi Moriguchi,§ Kunihiro Yamagata,* and Satoru Takahashi†‖¶

Departments of *Nephrology and †Anatomy and Embryology, Faculty of Medicine, ‖International Institute for Integrative Sleep Medicine (WPI-IIIS), and ¶Life Science Center of Tsukuba Advanced Research Alliance (TARA), University of Tsukuba, Ibaraki, Japan; ‡Department of Nephrology, Tokyo Medical University Ibaraki Medical Center, Inashiki, Ibaraki, Japan; and §Department of Medical Biochemistry, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan

ABSTRACT

We previously showed that the transcription factor Mafb is essential for podocyte differentiation and foot process formation. Podocytes are susceptible to injury in diabetes, and this injury leads to progression of diabetic nephropathy. In this study, we generated transgenic mice that overexpress Mafb in podocytes using the nephrin promoter/enhancer. To examine a potential pathogenetic role for Mafb in diabetic nephropathy, Mafb transgenic mice were treated with either streptozotocin or saline solution. Diabetic nephropathy was assessed by renal histology and biochemical analyses of urine and serum. Podocyte-specific overexpression of Mafb had no effect on body weight or blood glucose levels in either diabetic or control mice. Notably, albuminuria and changes in BUN levels and renal histology observed in diabetic wild-type animals were ameliorated in diabetic Mafb transgenic mice. Moreover, hyperglycemia-induced downregulation of Nephrin was mitigated in diabetic Mafb transgenic mice, and reporter assay results suggested that Mafb regulates Nephrin directly. Mafb transgenic glomeruli also overexpressed glutathione peroxidase, an antioxidative stress enzyme, and levels of the oxidative stress marker 8-hydroxydeoxyguanosine decreased in the urine of diabetic Mafb transgenic mice. Finally, Notch2 expression increased in diabetic glomeruli, and this effect was enhanced in diabetic Mafb transgenic glomeruli. These data indicate Mafb has a protective role in diabetic nephropathy through regulation of slit diaphragm proteins, antioxidative enzymes, and Notch pathways in podocytes and suggest that Mafb could be a therapeutic target.

J Am Soc Nephrol 25: ●●●–●●●, 2014. doi: 10.1681/ASN.2013090993

Diabetic nephropathy is the major cause of ESRD leading to dialysis. The earliest manifestations of the disease occur in the kidney glomerulus, and consequently, most attention to mechanisms has focused on altered responses of diabetic glomerular cells, especially mesangial cells and more recently, podocytes.[1] Podocytes are terminally differentiated epithelial cells that are key components of the selective permeability barrier of the glomerular basement membrane.[2] Podocytes seem to be quite susceptible to injury in diabetes,[3] and this injury leads to loss of podocytes, in part, by apoptosis.[4,5] This podocyte depletion seems to be a relatively early event in the evolution of diabetic nephropathy and predicts clinical progression of the disease.[6] The factors that lead to podocyte loss in diabetes are being actively investigated and include enhanced oxidative stress[5] and activation of the

Received September 20, 2013. Accepted February 18, 2014.

Published online ahead of print. Publication date available at www.jasn.org.

Correspondence: Dr. Naoki Morito, Department of Nephrology, Faculty of Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8575, Japan. Email: morito@md.tsukuba.ac.jp

renin-angiotensin-aldosterone system.[7] However, little is known about the specific mechanism.

Mafb is a basic leucine zipper transcription factor belonging to the large Maf family.[8] Maf family proteins share a conserved basic region and leucine zipper motif that mediates dimer formation and DNA binding to the Maf recognition element (MARE).[9] Analysis of Mafb-deficient mice has revealed that Mafb is essential for podocyte differentiation and its foot process formation.[10] The role of Mafb in kidney disease was not established, because Mafb-deficient mice die during the perinatal period. A recent study has revealed that multicentric carpotarsal osteolysis (MCTO) is caused by mutations clustering within the amino-terminal transcriptional activation domain of MAFB. MCTO is a rare skeletal dysplasia characterized by aggressive osteolysis, particularly affecting the carpal and tarsal bones, and it is frequently associated with progressive renal failure.[11] Zankl *et al.*[11] speculated that the pathogenesis of MCTO might be attributed to MAFB dysfunction in osteoclasts and podocytes. A decade ago, Fan *et al.*[12] identified a susceptibility locus for albuminuria by linkage analysis in diabetic KKT/a mice. Fan *et al.*[12] identified the *Mafb* gene in the vicinity of the susceptible locus and observed decreased expression of its product in the kidneys.

On the basis of these facts, we hypothesized that overexpression of Mafb in podocytes would prevent the development of diabetic nephropathy. In this study, we have generated transgenic mice that overexpress Mafb in podocytes using the Nephrin promoter and enhancer. Subsequently, we induced diabetic nephropathy in these transgenic mice.

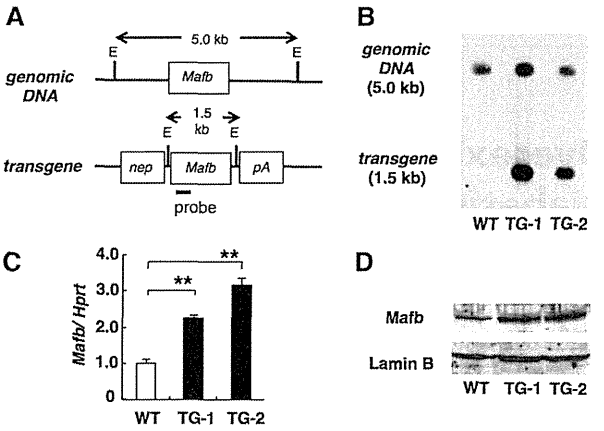

Figure 1. Mafb overexpression was observed in podocyte-specific Mafb transgenic kidneys. (A) Schematic of the Nephrin promoter-*Mafb* transgene construct. The *Mafb* cDNA was inserted into a vector that contained the human Nephrin promoter (*Nep*) and SV40 polyA (*pA*). The probe for Southern blot analysis, the restriction enzyme site (*EcoR*I [E]), and the predicted sizes of the endogenous gene and the transgene are indicated. (B) The 5.0-kb endogenous and 1.5-kb transgene fragments are shown from TG mice. (C) Analysis of *Mafb* mRNA in kidneys. The amount of *Mafb* mRNA in the samples from TG mice (TG-1 and TG-2) was higher than that in a sample from WT mice. Each bar represents the mean±SEM. $^{**}P<0.01$. (D) Western blot analysis of kidneys from WT and Mafb TG mice (TG-1 and TG-2). Expression of Mafb protein was elevated in TG mice. Hprt, hypoxanthine-guanine phosphoribosyltransferase.

RESULTS

Generation of Mafb Transgenic Mice That Express Mafb Specifically in Podocytes

To generate transgenic (TG) mice that express high levels of Mafb specifically in podocytes, the murine *Mafb* coding sequence was inserted into a vector that contained the Nephrin promotor/enhancer[13] (Figure 1A). We generated two TG mice lines on a C57BL/6J genetic background. Genomic DNA was analyzed by Southern blot to confirm the integrity and copy number of the transgene in each TG line. The *EcoR*I fragment that contained the *Mafb* transgene was 1.5 kb in length, whereas the corresponding fragment for the endogenous *Mafb* gene was 5.0 kb (Figure 1B). The results of densitometric analyses revealed that TG-1 and TG-2 each contained approximately four copies of the transgene (Figure 1B). These transgenes were stably transmitted to the progeny. To confirm that the transgenes were expressed, the levels of Mafb mRNA and protein in kidneys from TG mice were monitored by RT-PCR and Western blot (Figure 1, C and D). *Mafb* mRNA was overexpressed in kidneys of TG mice (Figure 1C). Consistent with this finding, Western blot analysis confirmed that the level of Mafb protein was also elevated. By the densitometry analysis, Mafb/LaminB ratio was 1.4 in TG-1, 1.6 in TG-2, and 1.0 in wild-type (WT) kidney (Figure 1D). We used TG-2 mice in the subsequent analysis.

Podocyte-Specific Mafb Overexpression Did Not Affect Blood Glucose, Insulin Levels, or Body Weight in Streptozotocin-Induced Diabetic Mice

Hyperglycemia was induced in 8-week-old mice by intraperitoneal injection of streptozotocin (STZ). After 12 weeks of hyperglycemia, the nonfasting blood glucose levels were 509.2±26.1 mg/dl in diabetic WT (WT STZ; n=10) and 537.7±29.8 mg/dl in diabetic Mafb TG (TG STZ) mice (n=10). There was no significant difference between blood glucose concentrations in WT STZ and TG STZ mice (Table 1). We also found that insulin concentrations and body weights were not significantly different between WT STZ and TG STZ animals (Table 1). We, thus, confirmed that Mafb overexpression did not affect diabetes induction by STZ.

Diabetic Nephropathy Was Ameliorated by Podocyte-Specific Mafb Overexpression

Next, we evaluated diabetic nephropathy in TG STZ mice. The level of urinary albumin in TG STZ mice was significantly lower than that of WT STZ mice at 20 weeks of age (Figure 2A). We

Table 1. Podocyte-specific overexpression of Mafb had no effect on body weight, blood glucose, or insulin levels in either diabetic or control mice

	WT CON (*n*=9)	TG CON (*n*=9)	WT STZ (*n*=10)	TG STZ (*n*=10)
Blood glucose, mg/dl (20 weeks)	170.7±7.0	175.3±18.3	509.2±26.1[a]	537.7±29.8[b]
Insulin, ng/ml (20 weeks)	1.05±0.08	0.99±0.08	0.34±0.03[a]	0.30±0.03[b]
Body weight, g (8 weeks)	22.6±0.8	21.9±0.7	22.7±0.6	22.4±0.7
Body weight, g (20 weeks)	28.8±1.1	30.1±0.7	24.2±0.8[a]	26.8±1.1[c]

Values represent the means±SEMs.
[a]$P<0.01$ versus WT CON.
[b]$P<0.01$ versus TG CON.
[c]$P<0.05$ versus TG CON.

also examined renal function in TG STZ mice by measuring BUN and serum creatinine as an index of renal function. The levels of BUN and serum creatinine were decreased in TG STZ mice compared with WT STZ mice, but the difference only reached significance in the case of BUN levels (Figure 2, B and C). Renal histopathology was also performed. In WT STZ mice, both glomerular hypertrophy and mesangial matrix expansion were observed (Figure 2D). Meanwhile, mesangial matrix expansion was less obvious in TG STZ mice (Figure 2D). Furthermore, the mean glomerular surface of TG STZ mice was significantly smaller than that of WT STZ mice (Figure 2E). We also found that renal interstitial damage was less obvious in TG STZ mice compared with WT STZ animals. (Figure 2, F and G).

Mafb Overexpression Prevented Hyperglycemia-Induced Mafb and Nephrin Depletion

To gain insight into the molecular mechanism by which diabetic nephropathy is ameliorated in Mafb TG mice, we monitored mRNA expression from *Mafb* and podocyte-specific genes in glomeruli using a quantitative RT-PCR assay (Figure 3A). In nondiabetic TG (TG CON) glomeruli, increased expression of *Mafb* was observed. *Mafb* expression was reduced after induction of hyperglycemia in WT STZ glomeruli. However, *Mafb* expression in TG STZ glomeruli remained greater than that observed in nondiabetic WT (WT CON) mice. We also observed increased expression of Nephrin gene (*Nphs1*) in TG CON glomeruli. Nephrin expression is reduced during STZ-induced experimental diabetic nephropathy.[14,15] Hyperglycemia-induced reduction of *Nphs1* expression was also observed in WT STZ mice but partially mitigated in TG STZ glomeruli. We did not detect significant changes in the levels of expression of *Nphs2* (Podocin) or *Podxl* (Podocaryxin) (Figure 3A).

Expression of the podocyte proteins Mafb, Nephrin, and Podocin was examined by semiquantitative immunofluorescence analysis (Figure 3, B–D). In WT STZ mice, Mafb expression was significantly decreased compared with the level observed in WT CON mice (Figure 3B). TG STZ mice, in contrast, exhibited no significant difference in Mafb expression compared with their TG controls. Similarly, Nephrin expression was significantly reduced in WT STZ mice compared with WT CON mice, whereas Nephrin levels did not significantly differ between TG CON and TG STZ mice (Figure 3C). We did not observe changes in Podocin expression (Figure 3D).

Mafb Directly Regulates Nephrin

We observed a significant reduction in the expression of *Nphs1* (Nephrin) mRNA in WT STZ mice, which was mitigated in TG STZ animals, suggesting a role for Mafb in the regulation of Nephrin mRNA expression. A 186-bp fragment of the human Nephrin promoter is capable of directing podocyte-specific expression of a β-galactosidase transgene when placed in front of a heterologous minimal promoter in TG mice.[16] In a separate paper, Moeller *et al.*[17] were able to show podocyte-specific expression using 1.25 kb of the proximal murine Nephrin promoter. We found that this Nephrin promoter region contains a half-MARE (TGCTGA) that is highly conserved between human and mouse (Nephrin-MARE) (Figure 4A). Therefore, we hypothesized that Mafb directly binds the Nephrin-MARE and activates Nephrin transcription. To test this hypothesis, we transfected a firefly luciferase reporter plasmid construct, which contained the murine Nephrin promoter region,[18] along with the Mafb expression plasmid into 293T cells. As expected, the reporter was activated by coexpression of Mafb in a dose-dependent manner (Figure 4B). However, luciferase reporter plasmid construct that contained mutations in the Nephrin-MARE within the Nephrin promoter was not activated by Mafb (Figure 4B). Electrophoretic mobility shift assays (EMSAs) showed that nonlabeled consensus MARE and Nephrin-MARE probes competed for binding to Mafb with the biotin-labeled consensus MARE or Nephrin-MARE probes (Figure 4C). These results suggest that Mafb directly regulates the transcription of Nephrin by interacting with the highly conserved Nephrin-MARE site in its promoter region.

Mafb Overexpression Decreased Oxidative Stress by Gluthatione Peroxidase Elevation in Diabetic Glomeruli

Podocytes are known to be susceptible to oxidative damage, and diabetes increases oxidative stress.[1,5] We evaluated oxidative stress by measuring the urinary excretion of 8-hydroxy-2-deoxyguanosine (8-OHdG) as a marker for oxidative DNA

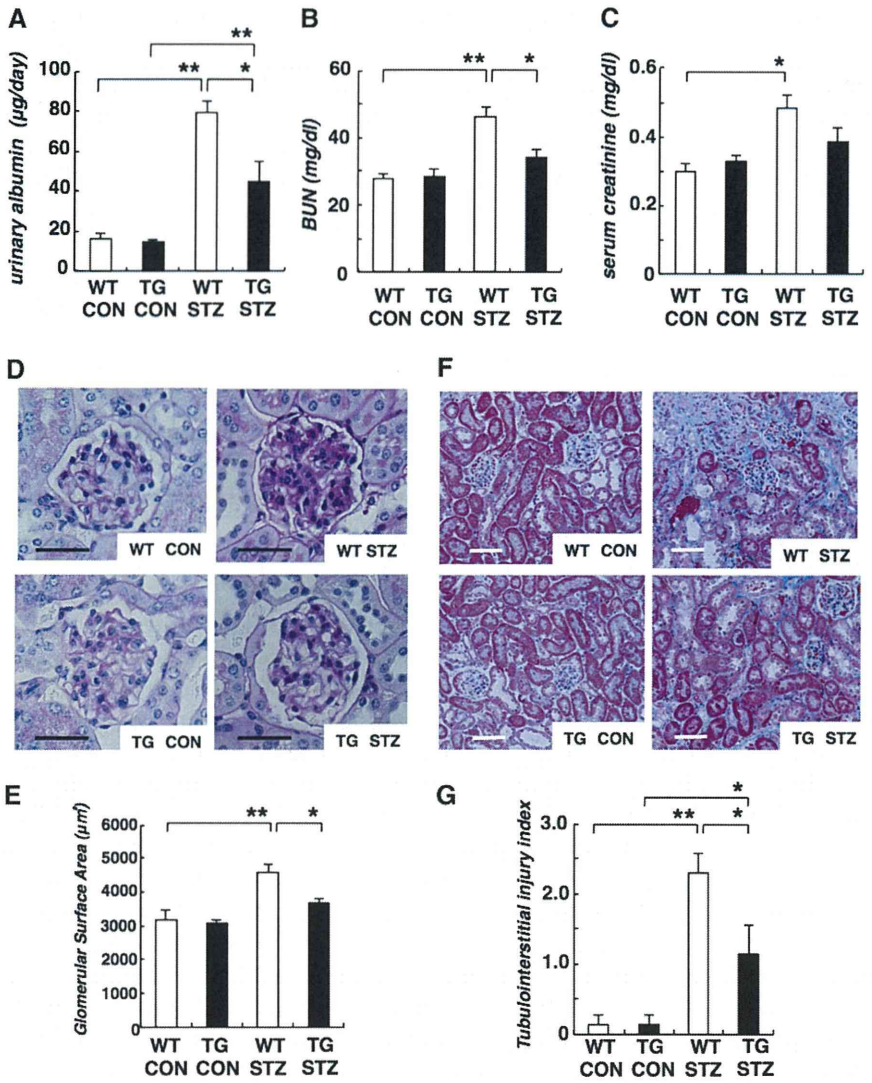

Figure 2. Diabetic nephropathy was improved in Mafb TG mice. (A) Urinary albumin, (B) BUN, and (C) serum creatinine were suppressed in TG STZ mice compared with WT STZ animals. (D) Periodic acid–Schiff-stained glomeruli. Glomerular hypertrophy and mesangial matrix expansion, which were observed in WT STZ mice, were ameliorated in TG STZ mice. Scale bar, 50 μm. (E) The mean glomerular surface area in TG STZ mice was smaller than that in WT STZ mice. (F) In the Masson's trichrome staining, the blue area, which shows fibrosis, was smaller in TG STZ kidney compared with WT STZ kidney. Scale bar, 100 μm. (G) Tubulointerstitial injury index in TG STZ mice was ameliorated compared with WT STZ animals. Values represent the means±SEMs (n=7 mice per group). *P<0.05; **P<0.01.

damage *in vivo*. After diabetes induction, urine 8-OHdG was increased in WT STZ mice. The increased 8-OHdG excretion was prevented in TG STZ animals (Figure 5A). Maf (c-Maf), a homolog of Mafb, is a transcriptional regulator of gluthatione peroxidase 3 (Gpx3), and it modulates the antioxidative pathway in the renal proximal tubule.[19] In addition, Gpx3 is also expressed in isolated glomeruli.[20] Recent data suggested that Gpx3 is produced by podocytes and interacts with podocin.[21] Moreover, glomerular Gpx is decreased in patients with FSGS and rats with puromycin aminonucleoside-induced nephropathy.[22] These facts suggest that podocytes Gpx might play a protective role against oxidative stress in diabetic nephropathy. Of the known Gpx isoforms, Gpx1 and Gpx4 are readily found in the kidney, whereas Gpx2 and Gpx5 are not.[23] RT-PCR analysis revealed decreased *Gpx3* and *Gpx4* expression in the WT STZ glomeruli (Figure 5B). In TG CON glomeruli, *Gpx3* expression was increased approximately 2-fold. Diabetes induction reduced Gpx3 expression to a similar overall percentage, although the level in TG STZ was similar to that in WT CON glomeruli. Decreased *Gpx4* expression was also seen in WT STZ glomeruli. However, there was no difference between *Gpx4* expression in WT STZ and TG STZ glomeruli. We could not detect obvious changes in *Gpx1* expressions. STZ causes a reduction in Gpx activity in both WT and TG mice, but the level in TG STZ mice is, nevertheless, similar to that in WT CON mice (Figure 5C).

Podocyte-Specific Mafb Overexpression Altered Glomerular Notch Signaling in Diabetic Mice

Translocation and/or overexpression of *MAFB* have been observed in some cases of human multiple myeloma, and *NOTCH2* was identified as a primary *MAFB* target gene in multiple myeloma cell lines.[24] Recent studies described increased podocyte Notch activity in proteinuric kidney diseases and suggest its contribution to the pathogenesis of proteinuria and diabetic nephropathy.[25,26] In this study, we investigated glomerular Notch pathways in diabetic Mafb TG mice (Figure 6). We found that glomerular Notch2 expression was increased in TG CON mice. However, the expression of other Notch subtypes was similar in WT and TG CON glomeruli. Increased expression of Notch1, Notch2, and Notch4 was observed in WT STZ glomeruli. Moreover, Notch1 elevation was mitigated in TG STZ glomeruli. Meanwhile, Notch2 expression was further potentiated after diabetes induction (Figure 6). There was no difference between Notch4 expression in WT STZ and TG STZ glomeruli. The expressions of Notch ligands (Jag1 and Dll1) and Notch target genes (Hes1 and Hey1) were elevated in WT STZ glomeruli. The fold induction of these genes was reduced in TG STZ glomeruli (Figure 6).

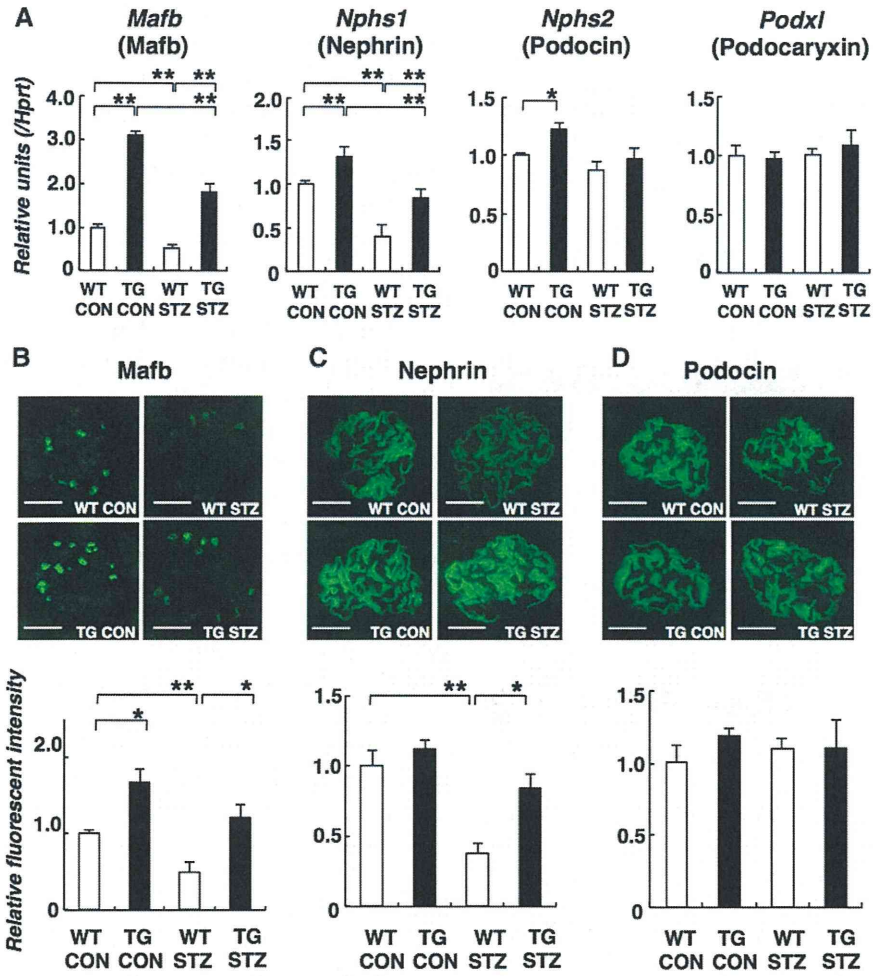

Figure 3. Hyperglycemia-induced Mafb and Nephrin depletion was prevented in Mafb TG mice. (A) Quantitative RT-PCR analysis. *Mafb* and *Nphs1* elevation was observed in TG CON glomeruli. Reduced expression of *Mafb* and *Nphs1* in WT STZ glomeruli was ameliorated in TG STZ mice. Significant changes in *Nphs2* and *Podxl* (Podocaryxin) expression were not observed. Analysis of (B) Mafb, (C) Nephrin, and (D) Podocin expression by immunofluorescence. Scale bar, 50 μm. Graphs depictin semiquantitative analysis by immunofluorescence. Mafb overexpression was observed in TG CON glomeruli. Reduction in glomerular Mafb and Nephrin expression induced by diabetes was mitigated in TG STZ mice. Podocin expression was unchanged. Values represent the means±SEMs (n=4 mice per group). *P<0.05; **P<0.01.

Mafb Induces Gpx3 and Notch2 Expression in Cultured Podocytes

We observed upregulation of Gpx3 and Notch2 in Mafb TG glomeruli, suggesting a role for Mafb in the regulation of Gpx3 and Notch2 expression. It was reported that c-Maf induces Gpx3 in renal tubule epithelial cells,[19] and c-Maf or Mafb induces Notch2 expression in myeloma cells.[24] However, there is no proof that Mafb regulates Gpx3 and Notch2 expression in podocytes. Therefore, we transfected murine podocytes with an Mafb expression vector or performed a mock transfection as a control. We observed elevated Gpx3 and Notch2 expression in Mafb-transfected podocytes by RT-PCR (Figure 7A) and Western blot analyses (Figure 7, B and C).

We also observed downregulation of Mafb and Gpx3 and upregulation of Notch2 in diabetic glomeruli. Additionally, we cultured Mafb-transfected and nontransfected podocytes in high glucose conditions (30 mM glucose). However, we did not observe any apparent change in the relative levels of Mafb, Gpx3, or Notch2 expression in cells cultured in media containing normal glucose (5.5 mM) versus high glucose (30 mM) for 72 hours (data not shown). Because hyperglycemia in STZ-treated diabetic mice persisted for 12 weeks, the difference between the *in vivo* and *in vitro* analyses might have arisen owing to the high glucose duration.

Overexpression of Mafb Restores Podocyte Depletion and Apoptosis Induced by Diabetes

Loss of podocytes contributes importantly to the pathogenesis of diabetic nephropathy.[1] We assayed the podocyte numbers by counting glomerular nuclei staining positively for Wilms' tumor 1 (WT1). The number of podocytes per glomerular section was reduced in WT STZ mice compared with WT CON animals. In contrast, podocyte depletion was mitigated in TG STZ mice (Figure 8, A and B).

Podocyte apoptosis has been identified as one cause of podocyte depletion in experimental kidney diseases in mice.[3–5] To determine whether the reduction in the apparent number of podocytes was related to increased cell death, sections were subjected to terminal deoxynucleotidyl transferase-mediated digoxigenin-deoxyuridine nick-end labeling (TUNEL) staining. Apoptotic glomerular cells were rare in glomerular sections from control mice. TUNEL-positive glomerular cells were increased in WT STZ mice, and this induction of cell death was significantly decreased in TG STZ mice (Figure 8C). We also confirmed that apoptotic glomerular cells were WT1-positive, indicating that they were podocytes (Figure 8D).

DISCUSSION

We previously showed that Mafb is essential for differentiation and foot process formation of podocytes.[10] A recent study