Journal of Nephrology (2021) 34:597–601
https://doi.org/10.1007/s40620-020-00770-9

CASE REPORT

Identical twins with idiopathic membranous nephropathy

Tian Tao[1] · Jue Wang[2] · Song Lei[3] · Zhangxue Hu[1]

Received: 30 March 2020 / Accepted: 3 June 2020 / Published online: 10 June 2020

Abstract
The pathogenesis of idiopathic membranous nephropathy is associated with autoantibodies, most often against the phospholipase A2 receptor (PLA2R) and with genetic factors, especially those involving human leukocyte antigen (HLA) genes. Idiopathic membranous nephropathy is not a typical inherited Mendelian disorder. Reports of idiopathic membranous nephropathy in twins are rare. Herein, we report on two twin sisters diagnosed with PLA2R-associated idiopathic membranous nephropathy. We identified the HLA-DRB1*0301, HLA-DRB1*1501, and HLA-DQB1*0602 alleles in the twin sisters, which were reported as independent risk alleles for idiopathic membranous nephropathy in the Asian population. This case report provides novel evidence for the role of predisposing HLA alleles in the pathogenesis of idiopathic membranous nephropathy.

Keywords Idiopathic membranous nephropathy · Twins · HLA genes · Anti-PLA2R antibody

Introduction

Idiopathic membranous nephropathy (IMN) is one of the most common causes of adult nephrotic syndrome. A pathologic feature of IMN is the deposition of immune complexes in the glomerular subepithelial space. The M-type phospholipase A2 receptor (PLA2R) has been identified as a major antigen in patients with IMN [1]. Circulating autoantibodies to PLA2R are found in 70–80% of patients with IMN and are associated with disease activity [2].

Studies have reported an association between human leukocyte antigen (HLA) class II and IMN [3–6]; however, studies in twins with IMN are rare. Herein, we report the case of a pair of Chinese twin sisters who were diagnosed with IMN. One patient was diagnosed with PLA2R-associated IMN. HLA gene screening revealed that both twins shared risk loci in HLA-DRB1 and HLA-DQB1. This study provides a novel evidence for the role of HLA in the pathogenesis of IMN.

Case presentation

Case 1

A 16-year-old girl was admitted to our hospital in June 2011 with a complaint of edema in the lower extremities for 4 months. No arthralgia, facial erythema, rash, hearing loss, or vision loss were noted. The patient's laboratory test results are shown in Table 1. Serum samples were positive for anti-PLA2R antibody (the detailed method used was previously reported [7]). Renal biopsy revealed the presence of tiny spikes along the glomerular basement membrane (GBM) (Fig. 1a, b). Immunofluorescence microscopy revealed granular deposits of IgG (3+) and C3 (+) along the glomerular capillary wall. Furthermore, the glomerular capillary positively stained for PLA2R (Fig. 1c). The results for thrombospondin type-1 domain-containing 7A (THSD7A) staining were negative. Electron microscopy (EM) revealed irregular thickening of the GBM with subepithelial electron-dense deposits (Fig. 1d). The patient was diagnosed with PLA2R-associated IMN. Therapy using a combination of prednisone and cyclophosphamide was initiated. Urinary protein levels returned to a normal range after 5 months. Till 2020, she has remained in remission with normal renal function.

✉ Zhangxue Hu
hzxawy@scu.edu.cn

[1] Department of Nephrology, West China Hospital, Sichuan University, Guoxue ally 37#, Wuhou District, Chengdu City, Sichuan Province, China

[2] Clinical Transfusion Research Center, Institute of Blood Transfusion, CAMS & PUMC, Chengdu, Sichuan, China

[3] Department of Pathology, West China Hospital, Sichuan University, Chengdu city, Sichuan Province, China

Table 1 Laboratory examination of the female twins

Patient	Case 1	Case 2
Proteinuria (g/24 h)	1.32	1.13
Hematuria (/HP)	8	4
ALB (g/dL)	3.93	3.53
Scr (mg/dL)	0.64	0.60
ANA	–	1:100
Anti-dsDNA	–	–
Tumor marker*	–	–
HBsAg	–	–
TP (ELISA)	–	–
Urinary mercury/Cr (μmol/mmol)#	0.021	0.015
Anti-GBM	–	–
ANCA	–	–
RF (IU/ml)	< 20	< 20

ALB serum albumin; *Scr* serum creatinine; *TP* treponema pallidum; *Cr* creatinine; *ANCA* anti-neutrophil cytoplasmic antibodies; *RF* rheumatoid factor

*Tumor markers include: CA15-3, CA19-9, CA-125, and CA72-4

#The normal range of urinary mercury/Cr is 0–2.25 μmol/mmol

– Negative or normal

Case 2

The second patient was the twin sister of the first case. The patient came to our hospital at the same time as Case 1. She presented with pretibial edema for 3 months. The patient's laboratory test results are listed in Table 1. Serum samples tested negative for anti-PLA2R antibody. Renal biopsy was performed. Light microscopy revealed the presence of spikes along the GBM (Fig. 1e). Immunofluorescence microscopy revealed granular deposition of IgG (2+) and C3 (+) along the GBM. In addition, the glomerular capillary was negative for PLA2R and THSD7A staining. EM revealed irregular thickening of the GBM with subepithelial electron-dense deposits (Fig. 1f). The patient was diagnosed with IMN and was treated with prednisone (50 mg/d) and mycophenolate mofetil (1.5 g/d) for 2 months before admission. Based on negative results for anti-PLA2R antibody in the serum and PLA2R staining in the glomeruli, the doses of prednisone and mycophenolate mofetil were soon tapered. Urinary protein levels gradually returned to a normal range over the next month. The patient has remained in remission since then.

Methods

DNA extraction

This study was performed according to the Declaration of Helsinki. After obtaining informed consent, blood samples were collected from the twin sisters and their family members (mother and brother). Genomic DNA was obtained using the Maxwell 16 Blood DNA Purification Kit (Promega, Madison, Wisconsin, USA).

Detection of human leukocyte antigen risk alleles

We performed HLA-DRB1 and HLA-DQB1, two risk alleles of IMN, genotyping of the family members via sequencing. The primers for HLA-DRB1 and HLA-DQB1 PCR-SBT are reported in the studies by Sayer DC et al. and van Dijk et al. [8], respectively. The HLA typing methods were validated in our laboratory by typing 48 DNA panels using commercial HLA typing kits (SeCore, Invitrogen; Brown Deer, Wisconsin, USA). Samples for proficiency testing were distributed by the University of California, Los Angeles.

Results

The twin sisters and their mother were found to have the same alleles (HLA-DRB1 *0301/*1501 and HLA-DQB1*0201/*0602). Furthermore, DRB1*1501-DQB1*0602 and DRB1*0301-DQB1*0201 were linked, respectively. The twins' younger brother was found to have HLA-DRB1*0101/*1501 and HLA-DQB1*0501/*0602.

Discussion

IMN is an organ-specific autoimmune disease caused by antibodies directed against target antigens located on the glomerular podocyte. Two podocyte proteins serving as autoantigens in patients with IMN have been identified in the past decade: PLA2R and THSD7A. PLA2R can be detected in glomerular podocytes and subepithelial immune deposits. Autoantibodies against PLA2R account for up to 80% of all cases of IMN. An increase in circulating anti-PLA2R antibody levels precedes proteinuria, while a decrease in circulating anti-PLA2R antibody levels precedes a decline in proteinuria [9, 10]. IMN with PLA2R deposition is termed as PLA2R-related membranous nephropathy. A few PLA2R single nucleotide polymorphisms (SNPs) have been reported to increase susceptibility to IMN [3, 11]. However, Coenen et al. did not show any specific coding mutation in 95 patients with IMN via direct sequencing of PLA2R1 exons and splice sites [12].

Many studies have shown a strong association between HLA polymorphisms and IMN since the 1970s. In earlier decades, HLA-DR3 presented the greatest risk of IMN. However, with the development of genotyping techniques, precise HLA loci have been revealed. DRB1*0301 is strongly associated with IMN in British [13] and Chinese

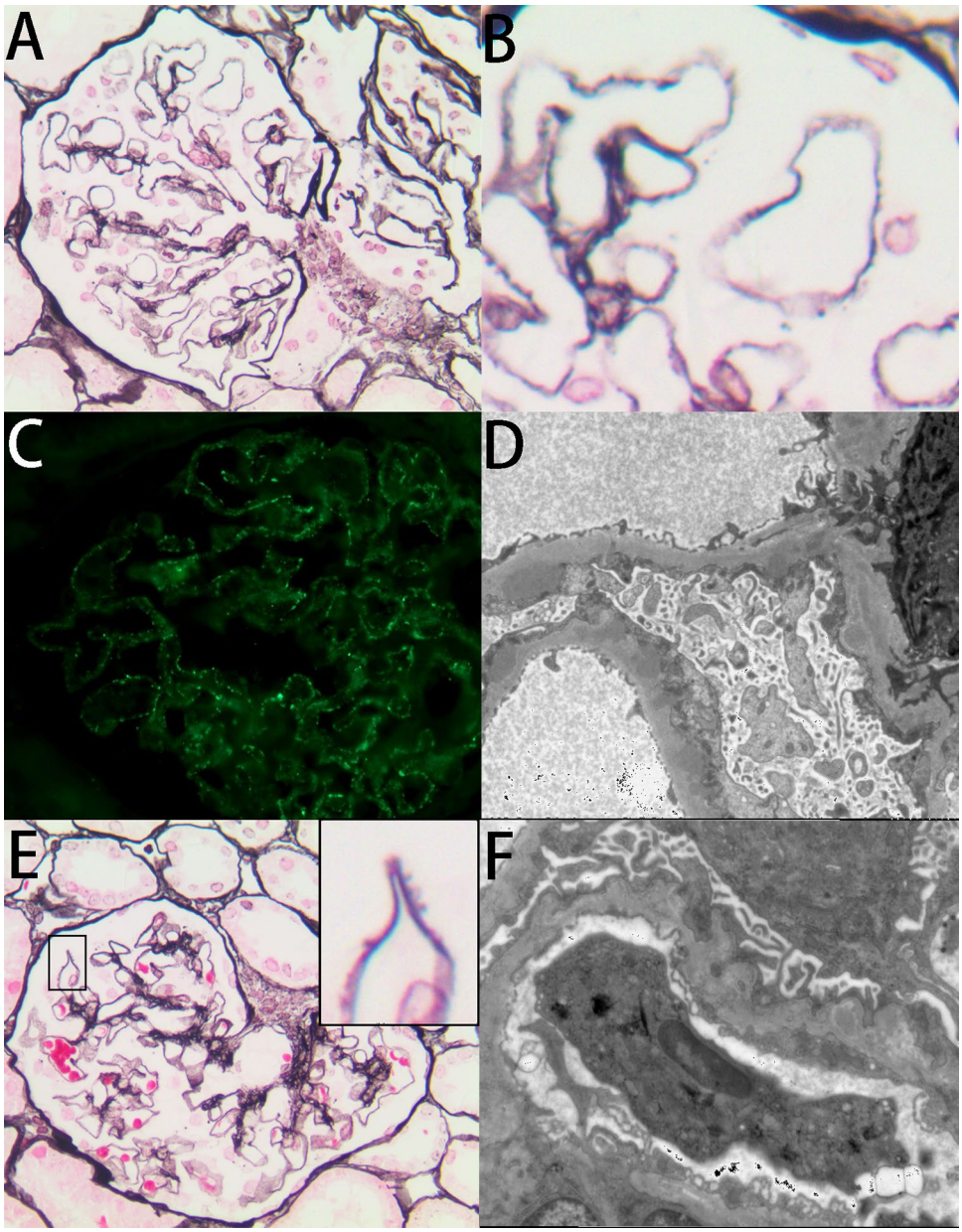

Fig. 1 Renal pathologic profiles of the twin sisters. **a** Shows the presence of tiny spikes along the glomerular basement membrane in Case 1(PASM, ×400); **b** shows the magnified spikes of **a**; **c** shows granular, positive PLA2R staining along the glomerular capillary wall (immunofluorescence, ×400) in Case 1; **d** shows the presence of subepithelial electron-dense deposits under electron microscopy (×12,000) in Case 1. **e** Shows spikes along the glomerular capillary wall in Case 2, with a partial enlarged view of the spikes (PASM, ×400). **f** Shows subepithelial electron-dense deposits under electron microscopy (×12,000) in Case 2

populations, with an allelic odds ratio of 3.96 [4, 14]. A genome-wide association study, which included three European cohorts, identified HLA-DQA1 (rs2187668) and the PLA2R (SNP rs4664308) as susceptible genomic loci in patients with IMN; the risk rate of HLA-DQA1 was five times higher than that of PLA2R [3]. Indeed, HLA-DQA1 (rs2187668) was once established as a tag SNP for DRB1*0301 in a European study [15]. The haplotype B*0801-DRB1*0301-DQA1*0501-DQB1*0201 is highly conserved in Northern European populations. Recently, DRB3*0202 was reported as an independent risk factor of PLA2R-associated IMN in the Chinese population, with an allelic odds ratio of 17.7 [5]. It is notable that DRB3*0202 resides on the same haplotype as DRB1*0301. All of the above finding support DRB1*0301 as a primary independent risk factor of IMN.

HLA-DRB1*1501 is another highly independent risk factor of IMN in the Han Chinese population, with an allelic odds ratio of 4.65 [4]. Genotype–phenotype correlation analyses revealed that DRB1*1501 was strongly associated with a younger age of disease onset. Along with DRB1*1501, DQB1*0602 has also been identified as a risk factor of IMN in a Japanese cohort, with an allelic odds ratio of 3.56 [6]. Additive effects exist in the risk of IMN among individuals with risk alleles of PLA2R1, HLA-DRB1*15:01, and DQB1*06:02, although HLA-DRB1*15:01-DQB1*06:02 is a common haplotype in the Japanese population. Protein homology modeling studies have revealed that risk HLA

alleles might participate in formation of the peptide-binding pocket of the HLA-DRβ1 chain, interacting with circulating anti-PLA2R1 antibodies [4].

IMN is not a typical hereditary disease in Mendelian terms, and studies undertaking twins as the study population are rare. Short et al. [16] reported three pairs of brothers with IMN. Among them, a pair of male twins possessed HLA antigens (B8, DR3). Vangelista et al. [17] described two sets of twin brothers who shared the same HLA antigens (A9 (23), 32, B35, DR3, and DR5). Guella et al. [18] reported male twins with IMN who shared HLA-DR3, B8, and DQ2. Other relatives who shared the same HLA antigen and who lived with the twins since childhood did not develop the disease. All studies conducted on twins with IMN did not reveal the IMN-related antigen and did not perform precise HLA genotyping.

This case was the first study in twins to assess PLA2R-associated IMN using HLA genotyping. Case 1 was a typical case of PLA2R-associated IMN with circulating PLA2R and its deposition in the glomeruli [19]. Case 2 was treated with prednisone and mycophenolate mofetil for 2 months before renal biopsy. Then she reached complete remission within 1 month. The fact that serum samples were negative for anti-PLA2R antibody should not overrule the relationship between PLA2R and IMN in Case 2. After all, these two patients are twins and experienced IMN simultaneously. HLA genotyping revealed that the twins held multiple risk alleles of IMN: HLA-DRB1*0301, DRB1*1501, and DQB1*0602. The summed HLA allele odds ratios may reach 65.6 in the East Asian population. Considering the interaction between PLA2R SNPs and HLA alleles, these twins were at a high risk of IMN. On the other hand, the twins' younger brother was at a reduced risk (HLA-DRB1*1501 and HLA-DQB1*0602) and did not develop the disease. Notably, IMN occurred at the same age in the twins but did not develop in the mother, despite identical haplotypes. Furthermore, other risk alleles that contribute to the occurrence of IMN, such as PLA2R1, nuclear factor kappa B subunit 1, and interferon regulatory factor 4, were not sequenced [20], and the mother only shares half of the genome of her children. Precipitating events, such as environmental, infectious, toxic factors, may trigger the disease. The twins did not take any medication, and there was no evidence of infection. Normal urinary mercury excretion rate excluded mercury poisoning. Thus, environmental factors may have contributed to the onset of the disease in the twins. Long-term exposure to air pollution, particularly to high levels of fine particulate matter of < 2.5 mm ($PM_{2.5}$), has been associated with an increased risk of membranous nephropathy in China [21]. $PM_{2.5}$ may be inhaled into the lung, resulting in the activation of inflammation, induction of epitope exposure, and activation of IMN. However, detailed mechanisms need to be further investigated.

Because the twin sisters were diagnosed in 2011, we had not performed PLA2R gene screening or full genomic sequencing of the HLA in the twin sisters.

Conclusion

We presented a case of Chinese female twins with PLA2R-associated IMN who expressed HLA-DRB1*0301, HLA-DRB1*1501, and HLA-DQB1*0602. This was the first study that was conducted in twins to examine PLA2R-associated IMN. The study adds novel evidence regarding the role of risk HLA alleles in the pathogenesis of IMN.

Author contributions TT and ZH were the physicians diagnosing and treating the patients in this report. JW was responsible for detection of human leukocyte antigen risk alleles. Song Lei was responsible for the pathologic results by electron microscopy. All authors participated in discussions about the manuscript and approved the final version.

Funding Support by grants from the National Natural Science Foundation of China (Grant number: 81470916) and Sichuan Province Key Technology R&D Program (Grant number: 2018SZ0122).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethics approval The authors state that they have obtained appropriate institutional review board approval and have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations.

Consent to participate Informed consent was obtained from all individual participants and legal guardians included in the study.

Consent for publication The participant signed informed consent regarding publishing their data and photographs.

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