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NEPHROLOGY - ORIGINAL PAPER

Circulating antibodies against M-type phospholipase A2 receptor and thrombospondin type-1 domain-containing 7A in Chinese patients with membranous nephropathy

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Abstract

Background M-type phospholipase A2 receptor (PLA2R) and thrombospondin type-1 domain-containing 7A (THSD7A) have recently been identified as target antigens for patients with idiopathic membranous nephropathy (IMN). The prevalence of PLA2R and THSD7A in the serum of MN patients deserves further investigation.

Methods Here, we studied the presence of anti-PLA2R and anti-THSD7A antibodies in patients with biopsy-proven IMN ($n=212$), secondary membranous nephropathy (SMN, $n=118$), and other kidney diseases ($n=84$). The progress of 49 IMN patients [anti-PLA2R(+), $n=27$; anti-THSD7A(+), $n=6$; anti-PLA2R(−) and anti-THSD7A(−) dual negative, $n=16$] who received immunosuppressive therapy was observed for 12 months. Serum concentrations of antibodies against PLA2R and THSD7A were detected using an indirect immunofluorescent assay.

Results One hundred fifty-two (71.7%) IMN patients and 11 (9.3%) SMN patients were identified as anti-PLA2R(+) anti-THSD7A(−). Five (2.4%) IMN patients and two (1.7%) SMN patients were identified as anti-THSD7A(+) anti-PLA2R(−). One of the IMN patients was identified as anti-PLA2R(+) and anti-THSD7A(+). The rate of partial remission was lower in anti-PLA2R(+) patients than in anti-PLA2R(−) patients 3 months ($P=0.045$) and 6 months ($P=0.006$) after immunosuppressive therapy. The rate of complete remission was lower in anti-PLA2R(+) patients than in anti-PLA2R(−) patients 12 months ($P=0.037$) after immunosuppressive therapy.

Conclusions The serum concentration of anti-PLA2R antibodies may be used as a sensitive and specific marker for diagnosing IMN. Immunosuppressive therapy is more effective for IMN patients who are anti-PLA2R(−) than for those who are anti-PLA2R(+).

Keywords PLA2R · THSD7A · Antibody · Membranous nephropathy

Introduction

Membranous nephropathy (MN), characterized by sub-epithelial immune deposits, complement activation, and alterations in podocyte structure and proteinuria, is the main cause of nephrotic syndrome in adults [1, 2]. While MN progresses slowly and about 30% of MN patients will go into remission spontaneously, end stage renal disease (ESRD) is expected in 30–40% of the MN patients [3]. There are two subtypes of MN, namely, idiopathic MN (IMN, etiology is unknown) and secondary MN (SMN, caused by various autoimmune diseases, infections, and malignancy) [4].

M-type phospholipase A2 receptor (PLA2R, about 180 kDa), which can bind to the phospholipase A2 enzymes (sPLA2), is in the mannose receptor family and is expressed on the membrane surface with a large extracellular portion [5]. In 2009, PLA2R was identified as an antigenic target for IMN (present in 70% of IMN patients) [6]. Soon after that, circulating concentrations of anti-PLA2R antibodies were proposed as an effective biomarker for monitoring the activity of IMN [7]. Meanwhile, in 2014, thrombospondin

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type-1 domain-containing 7A (THSD7A) was proposed as another IMN antigenic protein [8]. In that study, circulating anti-THSD7A antibodies were detected in about 10% of anti-PLA2R(−) IMN patients. Since then, many studies have reported the prevalence of these two autoantibodies in serum or in glomeruli of the kidney [9–11]. However, to date, very limited information is available in the literature regarding the occurrence of these two antibodies in Chinese MN patients. In the current study, the prevalence of anti-PLA2R and anti-THSD7A antibody detection was detected in Chinese IMN and SMN patients. Outcomes in Chinese IMN patients [with different antibody patterns; i.e., anti-PLA2R(+) anti-THSD7A(−), anti-PLA2R(−) anti-THSD7A(+), and anti-PLA2R(−) anti-THSD7A(−)] who received immunosuppressive therapy were observed as well.

Materials and methods

Patient cohorts

This was a cohort study conducted in Renmin Hospital at the Hubei University of Medicine (Shiyan, Hubei, China). IMN patients ($n=212$), SMN patients ($n=118$), and patients with other kidney diseases ($n=84$) in the period from January 1, 2016 to December 31, 2017 were selected for our study. All the patients were diagnosed by renal biopsy. Patients who received prior treatment with any immunosuppressive therapy were excluded.

Among this population, 49 IMN patients who received immunosuppressive therapy were observed for 12 months. The 24-h urine protein, PLA2R, and THSD7A levels were detected at the time of diagnosis and at 3, 6 and 12 months after immunosuppressive therapy.

Immunosuppressive therapy protocols

The treatment was intravenous pulse cyclophosphamide (CTX) plus oral prednisone. Briefly, CTX was intravenously administered at 600 mg/m^2 (maximum of 1 g) once every month for the initial 6 months and once every 2 months for during the later period. At the same time, oral prednisone was started at a dose of 1 mg/kg/day (maximum of 60 mg/day) for the first 8 weeks, gradually tapered by 5 mg every 2 weeks to 30 mg/day, then reduced by 5 mg every 4 weeks until a dose of 10 mg/day was maintained until the end of the 12-month therapy period.

Variables and definitions

The primary end points of the study included complete remission and partial remission. The secondary end points included the relapse rate and daily urinary protein levels. Partial remission was defined as a > 50% reduction in proteinuria compared to baseline and a persistent proteinuria of < 3.5 g/24 h. Complete remission was defined as persistent proteinuria of < 0.5 g/24 h. Relapse was defined as recurrence of proteinuria > 3.5 g/24 h or > 50% of the peak urinary protein levels in two consecutive urinalyses after an achievement of complete remission or partial remission.

The exposure variables were circulating antibodies of PLA2R and THSD7A tested at the initial time of follow-up.

Detection of circulating anti-PLA2R and anti-THSD7A antibodies

Serum concentrations of anti-PLA2R and anti-THSD7A antibodies were measured with a commercially available indirect immunofluorescence staining kit (Euroimmun, Luebeck, Germany) according to the manufacturer's instructions.

Statistical analysis

Data were analyzed with SPSS software (SPSS, Inc., Chicago, IL, version 19.0). Continuous parameters are shown as mean ± standard deviation (SD) and categorical parameters are shown as numbers and percentages. Quantitative parameters were analyzed by independent Student's *t* test or Kruskal–Wallis test. Categorical parameters were analyzed by chi-squared (χ^2) test or Fisher's exact test. Differences were considered significant at $P<0.05$.

Results

Patients enrolled and baseline characteristics

A total of 414 patients were enrolled, including 212 IMN patients, 118 SMN patients, and 84 patients with other kidney diseases. Among the patients with SMN, 51 cases were associated with lupus erythematosus, 45 cases were associated with hepatitis B, 2 cases were associated with psoriasis, 3 cases were associated with Sjogren syndrome, 2 cases were associated with hepatitis B and lupus erythematosus, and the other 15 cases were associated with malignancy. Among the patients with other kidney disease, 26 cases were associated with IgA nephropathy, 20 cases were associated with diabetic nephropathy, 15 cases were associated with minimal-change nephrotic syndrome, 10 cases were associated with mesangial proliferative glomerulonephritis, 8 cases were associated with focal segmental glomerular sclerosis, and the other 5 cases were associated with anaphylatic purpura nephritis.

The baseline information of the patients is presented in Table 1. The ages of the IMN, SMN, and control patients

Table 1 Baseline information of study population

	IMN	SMN	Other kidney disease	*P* value
No. of patients	212	118	84	–
Age, years (mean ± SD)	49.37 ± 9.63	48.47 ± 11.82	46.34 ± 11.05	0.459
Male (*n*)/female (*n*)	144/68	56/62	51/33	< 0.001
Proteinuria (g/24 h)	5.15 ± 2.01	4.72 ± 2.05	4.42 ± 2.27	0.069
Proteinuria > 3.5 g/24 h	163 (76.9%)	83 (70.3%)	53 (63.1%)	0.191
Albumin (g/L)	26.18 ± 4.32	26.80 ± 5.07	28.82 ± 4.47	0.244
Serum creatinine (μmol/L)	73.10 ± 20.61	74.16 ± 22.15	71.07 ± 20.61	0.664
Anti-PLA2R(+)	152 (71.7%)	11 (9.3%)	0	< 0.001
Anti-THSD7A(+)	5 (2.4%)	2 (1.7%)	0	0.688
Dual anti-PLA2R(+) and anti-THSD7A(+)	1 (0.5%)	0	0	0.455

P value represents IMN vs. SMN

were well matched (49.37 ± 9.63 years, 48.47 ± 11.82 years, and 46.34 ± 11.05 years, respectively). The gender ratios (male:female) in the IMN and SMN groups were 2.1:1 (144:68) and 1:1.11(56:62), respectively ($P < 0.001$). In patients with IMN and SMN, the mean urinary protein levels were 5.15 and 4.72 g/24 h, respectively ($P = 0.069$); the mean serum creatinine levels were 73.10 and 74.16 μmol/L, respectively ($P = 0.664$); and the mean albumin levels of were 26.18 and 26.80 g/L, respectively ($P = 0.244$). No difference ($P = 0.191$) was observed in the percentage of patients with proteinuria of ≥ 3.5 g/24 h between the IMN and SMN groups. The prevalence of anti-PLA2R(+) was significantly higher in IMN patients than in SMN patients ($P < 0.001$). In patients with IMN and SMN, the prevalence rates of anti-THSD7A(+) were 2.4% and 1.7%, respectively ($P = 0.688$), and the prevalence rates of dual anti-PLA2R(+) and anti-THSD7A(+) were 0.5% and 0, respectively ($P = 0.455$).

Prevalence of anti-PLA2R and anti-THSD7A antibodies in patients with MN

A bar chart of the detection of anti-PLA2R and anti-THSD7A antibodies in patients is shown in Fig. 1. Among the 212 patients with IMN, anti-PLA2R (but not anti-THSD7A) were detected in 152 (71.7%) patients, anti-THSD7A (but not anti-PLA2R) antibodies were detected in 5 (2.4%) patients, neither anti-PLA2R nor anti-THSD7A antibodies were detected in 54 (25.5%) patients, and both anti-PLA2R and anti-THSD7A antibodies were detected in 1 (0.5%) patient. Among the 118 SMN patients, anti-PLA2R (but not anti-THSD7A) antibodies were detected in 11 (9.3%) patients, anti-THSD7A (but not anti-PLA2R) antibodies were detected in 2 (1.7%) patients, and neither anti-PLA2R nor anti-THSD7A antibodies were detected in the other 105 (90.0%) patients. None of the SMN patients was positive for both anti-THSD7A and anti-PLA2R antibodies. Neither anti-THSD7A nor anti-PLA2R antibodies were detected in any of the 84 patients with other kidney diseases. The clinical features of all the patients with anti-THSD7A antibodies and SMN patients with anti-PLA2R antibodies are shown in Tables 2 and 3.

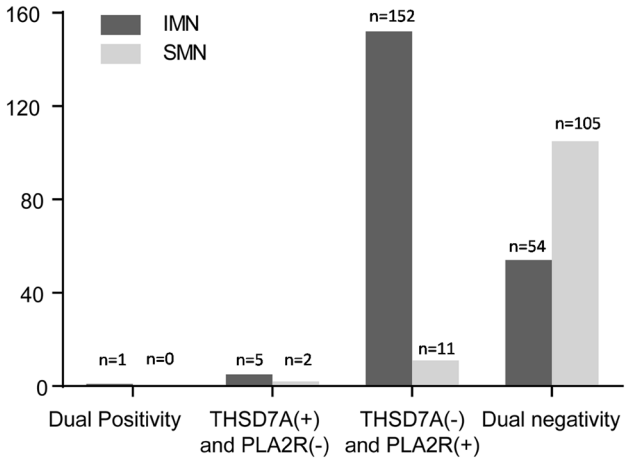

Fig. 1 Prevalence of anti-PLA2R and anti-THSD7A antibody detection in MN patients

Associations of serum anti-PLA2R antibodies and anti-THSD7A antibodies and the clinical findings among IMN patients

The baseline clinical characteristics of the IMN patients are listed in Table 4. IMN patients ($n = 212$) were divided into two groups: (1) antibody(+), representing patients with positivity for any of the antibodies; and (2) antibody(−), representing patients with no PLA2R-Ab and no THSD7A-Ab. Compared to antibody(−) patients, antibody(+) patients had higher levels of proteinuria ($P = 0.001$).

Table 2 Clinical features of patients with anti-THSD7A antibodies

No.	Age (years)	Gender	PLA2R	Diagnosis	Proteinuria (g/24 h)	Albumin (g/L)	Serum creatinine (μmol/L)
1	48	Male	+	IMN	7.8	28	43
2	63	Male	–	IMN	3.9	30	60
3	49	Female	–	IMN	5.5	25	77
4	49	Male	–	IMN	9.7	19	58
5	56	Male	–	IMN	4.6	29	129
6	43	Female	–	IMN	6.6	20	76
7	72	Male	–	SMN, lung cancer	4.9	26	119
8	54	Female	–	SMN, breast cancer	3.8	32	67

Table 3 Clinical features of SMN patients with anti-PLA2R antibodies

No.	Age (years)	Gender	Secondary factors	Proteinuria (g/24 h)	Albumin (g/L)	Serum creatinine (μmol/L)
1	43	Male	LN	3.7	26	87
2	36	Female	LN	6.4	18	65
3	31	Female	LN	4.3	24	97
4	29	Female	LN	5.6	21	110
5	49	Female	HBV	3.9	26	58
6	48	Male	HBV	4.2	27	89
7	52	Male	HBV	4.7	25	49
8	55	Male	HBV	5.2	19	54
9	65	Male	Prostatic cancer	3.8	29	104
10	52	Male	Colon cancer	4.4	33	125
11	48	Female	Sjogren syndrome	2.1	27	95

LN lupus nephritis, *HBV* hepatitis B virus

Table 4 Clinical baseline characteristics of IMN patients

	Antibody(+)	Antibody(−)	*P*
No. of patients	158	54	–
Age, years (mean ± SD)	49.13 ± 9.58	50.07 ± 9.91	0.535
Male/female	104/54	40/14	0.312
Proteinuria (g/24 h)	5.42 ± 2.12	4.34 ± 1.38	0.001
Proteinuria > 3.5 g/24 h	124 (78.5%)	39 (72.0%)	0.355
Albumin (g/L)	26.30 ± 4.36	25.83 ± 4.21	0.497
Serum creatinine (μmol/L)	73.69 ± 20.44	71.37 ± 21.38	0.478

P value represents antibody(+) vs. antibody(−)

Clinical follow-up

A total of 49 IMN patients who received immunosuppressive therapy for at least 12 months were observed. According to the exposure variables, the 49 IMN patients were divided into three groups: anti-PLA2R(+), $n = 27$; anti-THSD7A(+), $n = 6$; anti-PLA2R(−) and anti-THSD7A(−) dual negative, $n = 16$. Patients with dual anti-THSD7A(+) and anti-PLA2R(+) were included in the THSD7A-Ab(+) group, which had a very limited patient number. All the participants successfully accomplished the 12-month follow-up, and no case was lost.

The information in Table 5 was recorded at the initial time of follow-up and at 3, 6 and 12 months after immunosuppressive therapy. The levels of proteinuria decreased linearly after immunosuppressive therapy. The rate of partial remission was lower in anti-PLA2R(+) patients than in anti-PLA2R(−) patients at 3 months ($P = 0.045$) and 6 months ($P = 0.006$) after immunosuppressive therapy. The rate of complete remission was lower in anti-PLA2R(+) patients than in anti-PLA2R(−) patients at 12 months ($P = 0.037$) after immunosuppressive therapy. The partial remission rate and complete remission rate were not significantly different between the anti-THSD7A(+) patients and anti-PLA2R(−) patients at 3, 6 and 12 months after immunosuppressive therapy. After remission was induced, relapse was recorded in 3 of 27 (11.1%) anti-PLA2R(+) patients, in 1 of 6 (16.7%) anti-THSD7A(+) patients, and in 1 of 16 (6.3%) PLA2R-Ab(−) patients. No difference was observed in the relapse rate during the 12-month follow-up period among the groups.

Table 5 Changes in clinical characteristics of the study population during follow-up

	PLA2R-Ab(+) IMN	THSD7A-Ab(+) IMN	PLA2R-Ab(−) and THSD7A(−) IMN	P^a	P^b
No. of patients	27	6	16	–	–
Age, years	48.67 ± 5.41	51.33 ± 7.06	50.69 ± 11.74	0.524	0.901
Male/Female	18/9	4/2	12/4	0.735	0.696
Initial					
Proteinuria (g/24 h)	7.58 ± 1.36	6.35 ± 2.15	5.97 ± 1.30	< 0.001	0.615
PLA2R-Ab	27/27	1/6	–	–	–
THSD7A-Ab	–	5/6	–	–	–
At 3 months					
Proteinuria (g/24 h)	4.57 ± 0.72	3.92 ± 0.62	3.83 ± 0.95	0.006	0.842
PLA2R-Ab	23/27	1/6	–	–	–
THSD7A-Ab	–	4/6	–	–	–
Partial remission (*n*)	0/27(0%)	0/6 (0%)	3/16 (18.8%)	0.045	0.532
Complete remission (*n*)	0/27(0%)	0/6 (0%)	0/16 (0%)	–	–
At 6 months					
Proteinuria (g/24 h)	3.79 ± 0.93	3.52 ± 0.76	2.29 ± 1.17	<0.001	0.028
PLA2R-Ab	20/27	0/6	–	–	–
THSD7A-Ab	–	3/6	–	–	–
Partial remission (*n*)	7/27(25.9%)	2/6 (33.3%)	11/16 (68.8%)	0.006	0.178
Complete remission (*n*)	0/27(0%)	0/6 (0%)	1/16 (6.3%)	0.372	0.531
At 12 months					
Proteinuria (g/24 h)	3.09 ± 1.72	2.38 ± 1.71	1.70 ± 1.92	0.019	0.455
PLA2R-Ab	17/27	0/6	–	–	–
THSD7A-Ab	–	2/6	–	–	–
Partial remission (*n*)	15/27 (55.6%)	4/6 (66.6%)	8/16 (50.0%)	0.724	0.484
Complete remission (*n*)	2/27 (7.4%)	1/6 (16.7%)	6/16 (37.5%)	0.037	0.350
Relapse	3/27 (11.1%)	1/6 (16.7%)	1/16 (6.3%)	0.596	0.449

[a]PLA2R-Ab(+) IMN vs. PLA2R-Ab(−) and THSD7A(−) IMN

[b]THSD7A-Ab(+) IMN vs. PLA2R-Ab(−) and THSD7A(−) IMN

Discussion

Anti-PLA2R antibodies have been detected in 52–82% IMN patients in the literature [12–15]. In this study, anti-PLA2R was detected in 71.7% patients with IMN and in 9.3% patients with SMN. Similarly, the rate of anti-PLA2R(+) is about 72% in European patients [16]. Patients with IMN had an increased prevalence of anti-PLA2R(+) compared with SMN patients. Therefore, the circulating concentration of anti-PLA2R might be used as a sensitive marker for distinguishing IMN from SMN. An updated meta-analysis investigating the diagnostic accuracy of the serological PLA2R to differentiate IMN and SMN reported a pooled sensitivity and specificity of 68% and 97%, respectively [17].

According to Ronco et al. [18], anti-PLA2R antibodies are presented in 10–30% of SMN patients. Qin et al. [13] reported that anti-PLA2R antibodies could be detected in Chinese patients with lupus-, HBV-, and tumor-associated MN.

The presence of anti-PLA2R antibodies in SMN patients might result from the co-incidental simultaneous development of IMN and systemic diseases (e.g., sarcoidosis, malignancy, and psoriasis) [19–21]. In our study, among the 11 anti-PLA2R(+) SMN patients, 4 cases were associated with lupus, 4 cases were associated with HBV, 2 cases were associated with tumors, and the other 1 case was associated with Sjogren Syndrome. In SMN patients, anti-PLA2R antibodies might be derived by the underlying pathogenic mechanisms driving the disease or the ethnic group involved.

The prevalence of THSD7A in serum and glomeruli samples has been reported in many studies of MN since Tomas et al. [8] first reported that 15 of 154 IMN patients had circulating autoantibodies to THSD7A but not PLA2R [9–11]. Basically, THSD7A is present in 2.1–9.1% of IMN patients in existing studies. Iwakura et al. [9] reported that granular expression of THSD7A was detected in 9.1% of IMN patients in Japan. The differences between the current results and Iwakura et al.'s findings (both from Asian

populations) might be explained, at least in part, by the differences in staining methods and sample sources. Sharma et al. [22] reported that a total of 31 IMN patients were positive for THSD7A, for a prevalence of 2.4% among patients with IMN in the US and Europe. Jia et al. [23] detected THSD7A in 12 of 578 (2.1%) IMN patients. In the current study, anti-THSD7A antibodies were presented in 2.4% of the IMN patients and 1.7% of the SMN patients. Among the 59 anti-PLA2R(−) patients, 5 (8.5%) were identified as anti-THSD7A(+). These results suggest that the prevalence of anti-THSD7A antibodies was higher in anti-PLA2R(−) IMN patients than in anti-PLA2R(+) IMN patients. The potential use of anti-THSD7A in diagnosing IMN needs further study.

We identified one patient in our cohort who was positive for both anti-PLA2R and anti-THSD7A antibodies. This case is of great rarity, with only five cases reported in three papers to date [10, 23, 24]. For this particular patient, it is hard to comment on whether both PLA2R and THSD7A are pathogenic. It is possible that one of these antibodies was induced in an 'opportunistic' manner after exposure to the podocyte antigens. Also, we identified two anti-THSD7A(+) SMN patients, another situation that has been rarely reported. In the literature, only four cases of anti-THSD7A(+) SMN patients were reported [8, 23, 25]. Among these four cases, two patients were speculated to have two independent diseases (i.e., IMN and lupus or prostate cancer) [8], one patient had SMN associated with urinary bladder cancer [23], and the other patient was a 40-year-old woman who simultaneously suffered from a metastasized mixed adenoneuroendocrine carcinoma (MANEC) of the gall bladder (THSD7A was detected in both the gall bladder primary tumor and the serum). Furthermore, THSD7A antibodies were present in the corresponding lymph node metastases [25]. In our study, two patients were diagnosed with SMN associated with lung and breast cancers. Further investigations are needed to study the antigenic roles of THSD7A in tumor development and SMN. Overall, screening for malignancies is important in anti-THSD7A(+) MN patients.

Anti-PLA2R antibodies can bind to PLA2R, induce in situ formation of subepithelial deposits, damage podocytes and cause proteinuria. Therefore, we studied the relationship between the serum antibody level and proteinuria. Increased levels of proteinuria ($P = 0.001$) were recorded in antibody(+) patients compared to antibody(−) patients. Similar observations were made by Li et al. [26]. While these results suggest an association between the presence of anti-PLA2R/anti-THSD7A antibodies and the activity of clinical disease, the antigenic roles of THSD7A remain unclear. The next step is to study the antigenic roles of THSD7A in MN and to evaluate the potential use anti-THSD7A antibodies in monitoring the activity of MN.

The levels of proteinuria, anti-PLA2R and anti-THSD7A decreased linearly with time after immunosuppressive therapy. The PLA2R-Ab(−) IMN patients were more likely to experience partial remission at an early stage and more likely to experience complete remission after 12 months than the anti-PLA2R(+) patients. Among the anti-PLA2R(−), anti-PLA2R(+) and anti-THSD7A(+) patients, no difference was observed in the relapse rate during the 12-month follow-up period. The relapse rate will need to be studied with a longer follow-up time.

The antigenic roles of PLA2R and THSD7A in IMN have created a new research area for developing strategies to diagnose and even treat IMN. It is highly possible to diagnose and propose effective therapy methods for MN patients by simply detecting circulating levels of anti-PLA2R antibodies. Serologic anti-PLA2R antibodies may also be used as a sensitive marker for monitoring the immunological remission rate and evaluating the effectiveness of immunosuppressive therapy.

Further studies should focus not only on the presence and antigenic properties of PLA2R and THSD7A in MN patients, but also on the physiological functions of these molecules in healthy subjects (normally functional podocytes).

Conclusion

IMN patients with anti-PLA2R(−) were likely to experience partial remission at an earlier stage than those who were anti-PLA2R(+). Anti-PLA2R might be used as a sensitive and specific marker for distinguishing IMN from SMN in Chinese populations. The prevalence of anti-THSD7A(+) was low in IMN and SMN patients.

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Compliance with ethical standards

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

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee (the Ethics Committee of Hubei University of Medicine) and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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