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ORIGINAL PAPER

Intermediate filaments in the medial rectus muscles in patients with concomitant exotropia

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Abstract

Purpose Distribution of intermediate filament (IF) proteins in normal extraocular muscles (EOMs) showed that the EOMs differ significantly from the other muscles in the body with respect to their IFs composition, including desmin and nestin. The aim of the present study was to investigate the pathological changes in the medial rectus (MR) in patients with concomitant exotropia (XT).

Methods Forty-six MR muscle samples from 46 patients with XT were analyzed pathologically and processed for immunohistochemistry with specific antibodies against desmin and nestin.

Results Although most of MR muscles remained normal structures relatively, they presented high expression of desmin, and in contrast, nestin was absent in a large proportion of the MR muscles.

Conclusion Desmin, which is downregulated in normal EOMs, had high expression in MR muscles of patients with XT. Nestin, which is present in a high proportion of normal EOMs, was downregulated in MR muscles of patients with XT.

Keywords Concomitant exotropia · Extraocular muscles · Desmin · Nestin

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Introduction

Strabismus, including concomitant strabismus and incomitant strabismus, is one of the most common disorders in pediatric ophthalmology, with an estimated prevalence of 3–5% worldwide [1]. Concomitant strabismus is characterized by a constant angle of deviation in all fields of gaze, and the deviation amplitude remains the same with either eye fixating. Hitherto in this type of horizontal deviation, there was no characteristic pathological change detected in extraocular muscles (EOMs) and its neurological innervations. And, the horizontal rectus muscle path lengths are not significantly abnormal in concomitant strabismus [2]. So, in the absence of obvious structural abnormalities of the eye or brain, the etiology of concomitant strabismus remains unclear.

The normal EOMs differ from typical skeletal muscles at the cellular and molecular level, in particular regarding the composition of the major proteins determining contraction force and velocity, calcium transportation proteins, extracellular matrix proteins, and neuromuscular junction gangliosides [3–6]. So in the patients with concomitant strabismus, the pathological structures and protein levels are probably more different from the normal EOMs and typical skeletal muscles. Indeed, our previous study confirmed the abnormal expression of structural proteins in some of the 324 EOMs of 278 patients with concomitant strabismus (unpublished data). And,

we also found abnormal expression of myogenesis-related genes in EOMs of patients with concomitant strabismus [7]. Recent study on distribution of intermediate filament (IF) proteins in normal EOMs showed that the EOMs differ significantly from the other muscles in the body with respect to their IFs composition, including desmin and nestin [8].

In the present study, we investigated the pathological changes in the medial rectus (MR) muscles in patients with concomitant exotropia (XT), in order to identify whether there are special morphologic changes or expression of IF proteins in these muscles. Remarkably, we found changes in IFs composition of MR muscles with XT.

Materials and methods

Samples

The MR muscles were obtained from patients with XT during strabismic surgery at Zhongshan Ophthalmic Center, Guangzhou, China. A total of 46 MR muscles from 46 patients with XT were analyzed in this study. All of the muscle samples were obtained at least 2 mm from the insertion of each muscle into the globe, and the average muscle sizes dissected were 4–8 (6 ± 1.32) mm. All of the surgeries were taken by the same surgeon (Deng D.M.), and the force duction test was processed in all of the cases before the surgery in order to assess the restriction of ocular movements. Before the surgery, the clinical information of the individuals had been collected as shown in Table 1, including gender, duration of disease, angle of deviation, visual acuity, refraction, and function of binocular vision. None of the included cases was treated by Botox.

This study was performed in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained in every case from participants or their guardians before the collection of muscle samples. This study was approved by the Institutional Review Board of the Zhongshan Ophthalmic Center.

Masson's trichrome (MT) staining

The muscle samples were embedded in paraffin and processed for coronal sectioning at 10 μm thickness, as described previously [9]. The MR muscle samples were step-sectioned transversely at intervals of 200 μm. The sections were mounted on poly-L-lysine-coated slides, dried at 60 °C overnight, dewaxed with xylene, and gradually hydrated. MT stain was applied to dewaxed sections to visualize muscle and connective tissue constituents [10].

Immunohistochemical analysis

Antigen retrievals of the dewaxed sections were achieved by pressure-cooking in 0.01 mol/L citrate buffer for 15 min. Endogenous peroxidase was blocked by incubation with 3% hydrogen peroxide (H_2O_2) for 10 min. To reduce nonspecific binding, the sections were incubated with 20% normal goat serum for 10 min at 37 °C. Primary antibodies were incubated overnight at 4 °C, followed by rinsing with phosphate-buffered saline (PBS). Then, the slides were incubated for 10 min at room temperature with secondary antibodies and were stained for 10 min with 3,3′-diaminobenzidine tetrahydrochloride (DAB). Negative controls were subjected to the same procedure, except that the primary antibody was replaced by PBS.

All of the antibodies were purchased from BioVision in the USA (BioVision, Inc., Milpitas, CA, USA), and the finial dilutions were 1:50.

Semiquantitative evaluation of staining

The pathologist, who was unaware of the patients, independently examined the slides. For this study, only the cytoplasmic immunohistological staining was scored, and only the staining of myocytes was obtained. Staining intensity was categorized into four groups by comparing the staining intensity of muscle cells with that of vascular endothelial cells. The staining intensity was scored as 0 (no staining), 1 (faint yellow), 2 (brown-yellow), or 3 (dark yellow). The extent of staining was scored according to the proportion of positive cells in the muscle cells, as 0 (< 5%), 1 (5–25%), 2 (26–75%), or 3 (more than 76%). The final score of 0–6 was obtained by summing the two scores above, and the expression categories of desmin and nestin were defined as follows: − (0), + (1–2), ++ (3–4), and +++ (5–6).

Table 1 Clinical information of patients with concomitant exotropia

Patient	Gender	Age (year)		Deviation (°) with naked eyes				Visual acuity		Preoperative refraction (D)				BCVA		Binocular
		Surgery	Onset	Distance (OD)	Distance (OS)	Near (OD)	Near (OS)	OD	OS	SPH (OD)	CYL (OD)	SPH (OS)	CYL (OS)	OD	OS	Vision
X01	M	5	4	XT24	XT22	XT28	XT28	ND	ND	+ 0.50	+ 1.00 * 90	+ 0.25	+ 1.25 * 92	0.5	0.5	MS
X02	M	4.5	1	XT33	XT25	XT36	XT32	0.3	0.3	+ 1.00	+ 0.50 * 180	+ 1.50	0	0.7	0.9	MS
X03	M	5	3	XT16	XT19	XT20	XT20	1.0	1.2	+ 2.75	+ 0.50 * 80	+ 2.25	+ 0.25 * 80	1.0	1.0	MS
X04	F	8	3	XT12	XT17	XT21	XT25	0.3	0.4	− 1.25	− 0.75 * 175	0	− 2.50 * 171	1.2	1.0	NRC I
X05	M	12	4	XT12	XT20	XT18	XT24	1.0	1.0	− 1.00	− 0.75 * 175	− 0.25	− 0.75 * 175	1.0	1.0	NRC I
X06	F	12	1	XT40	XT43	XT41	XT42	1.5	1.5	0	− 0.50 * 165	0	0	1.5	1.5	MS
X07	F	9	2	XT29	XT32	XT23	XT26	1.5	1.5	0	+ 0.75 * 80	+ 0.50	+ 0.50 * 70	1.5	1.5	MS
X08	M	22	1	XT35	XT38	XT44	XT40	1.5	1.5	+ 0.75	0	0	+ 0.50 * 10	1.5	1.5	MS
X09	F	24	4	XT34	XT34	XT38	XT38	1.0	1.2	− 0.50	+ 1.25 * 85	− 0.50	+ 1.00 * 85	1.0	1.2	MS
X10	F	25	10	XT46	XT52	XT47	XT53	1.0	1.2	+ 0.25	− 0.50 * 15	0	+ 0.25 * 80	1.0	1.2	MS
X11	F	36	7	XT36	XT25	XT30	XT32	0.8	0.8	− 0.50	− 0.50 * 70	− 0.50	− 0.50 * 110	1.2	1.2	MS
X12	M	21	7	XT20	ND	XT28	ND	0.1	1.5	+ 4.00	+ 2.00 * 115	+ 1.00	+ 0.50 * 80	0.1	1.5	MS
X13	M	37	C	XT36	XT41	XT41	XT43	0.8	0.8	− 0.25	− 1.00 * 70	− 0.50	− 1.25 * 120	1.5	1.5	MS
X14	F	10	2	XT21	XT20	XT24	XT25	1.0	0.6	+ 1.00	+ 0.25 * 170	− 0.50	− 0.50 * 155	1.0	0.6	MS
X15	F	30	12	XT10	XT18	XT16	XT20	0.4	0.6	− 2.00	0	− 2.00	0	1.2	1.2	MS
X16	M	5	4	XT14	XT19	XT20	XT20	0.6	0.9	+ 1.00	+ 0.50 * 5	+ 1.50	+ 0.25 * 100	0.7	0.9	NRC I
X17	F	24	0.5	XT33	XT38	XT33	XT41	0.05	0.5	− 3.75	− 2.00 * 10	0	− 2.25 * 170	0.2	0.6	MS
X18	F	22	2	XT25	XT26	XT28	XT33	1.2	1.2	0	0	0	0	1.2	1.2	MS
X19	M	40	20	XT37	XT40	XT39	XT44	1.5	1.2	+ 1.25	0	+ 1.75	+ 0.50 * 170	1.5	1.2	MS
X20	F	7	2	XT20	XT24	XT22	XT27	1.2	1.2	+ 1.25	+ 0.25 * 90	+ 1.50	+ 0.50 * 75	1.2	1.2	MS
X21	M	11	8	XT12	XT12	XT21	XT21	0.7	0.7	− 0.50	0	− 0.50	0	1.0	1.0	MS
X22	F	20	16	XT25	XT27	XT34	XT38	1.0	1.2	+ 1.00	0	+ 1.75	0	1.0	1.2	MS
X23	M	28	4	XT43	XT37	XT46	XT48	0.08	0.1	− 3.75	− 1.50 * 175	− 2.50	0	1.0	1.0	MS
X24	F	6	1	XT20	XT21	XT24	XT27	0.7	0.7	+ 1.75	+ 0.50 * 150	+ 1.75	0	0.7	0.7	MS
X25	F	24	4	XT25	XT35	XT28	XT37	0.1	0.9	− 3.25	0	− 0.25	0	1.0	1.0	MS
X26	F	5	2	XT14	XT16	XT18	XT15	0.7	0.8	+ 3.00	0	+ 2.75	0	0.7	0.8	MS
X27	M	26	10	XT35	XT34	XT39	XT46	0.1	0.06	− 6.75	− 2.25 * 27	− 7.75	− 1.50 * 180	1.0	1.2	MS
X28	M	4	2	XT20	XT16	XT22	XT23	1.2	1.0	+ 1.50	+ 0.50 * 110	+ 1.50	0	1.2	1.0	MS
X29	M	7	4	XT15	XT18	XT16	XT18	0.3	0.2	− 1.75	0	− 2.00	0	1.0	1.0	MS
X30	M	13	10	XT21	XT15	XT25	XT26	0.2	0.2	− 4.00	− 1.25 * 170	− 4.00	− 1.00 * 180	1.0	1.0	NRC II

Table 1 continued

Patient	Gender	Age (year)		Deviation (°) with naked eyes				Visual acuity		Preoperative refraction (D)				BCVA		Binocular
		Surgery	Onset	Distance (OD)	Distance (OS)	Near (OD)	Near (OS)	OD	OS	SPH (OD)	CYL (OD)	SPH (OS)	CYL (OS)	OD	OS	Vision
X31	M	7	4	XT18	XT20	XT18	XT19	0.4	0.4	− 2.50	0	− 2.25	0	1.0	1.0	NRC II
X32	F	7	3	ND	XT24	ND	XT28	1.2	0.3	0	0	+ 1.25	+ 0.75 * 80	1.2	0.3	NRC I
X33	M	7	2	XT12	XT18	XT18	XT15	1.5	0.2	+ 1.50	+ 0.25 * 75	− 2.50	− 2.00 * 5	1.5	0.6	NRC I
X34	F	6	4	XT18	XT21	XT25	XT26	1.5	1.2	+ 1.75	0	+ 2.00	+ 0.50 * 50	1.5	1.2	MS
X35	M	13	1	XT19	XT20	XT20	XT21	0.6	0.7	+ 0.50	0	0	− 0.50 * 10	0.6	0.7	MS
X36	M	20	C	XT20	XT28	XT28	XT30	0.3	0.1	+ 7.00	+ 1.00 * 150	+ 6.25	+ 1.25 * 55	0.5	0.1	MS
X37	F	24	C	XT42	XT40	XT49	XT35	0.4	0.1	− 1.50	− 0.50 * 20	− 3.25	− 0.50 * 160	1.5	1.2	MS
X38	F	9	C	XT29	XT33	XT35	XT35	0.4	0.6	+ 0.75	− 3.25 * 170	+ 1.75	− 3.50 * 175	1.0	1.0	MS
X39	M	3	1	NA	NA	XT35	XT33	NA	NA	+ 0.75	+ 0.25 * 90	+ 0.50	+ 0.50 * * 80	NA	NA	NA
X40	F	21	C	XT27	XT27	XT33	XT35	0.3	0.7	− 1.50	− 0.75 * 20	+ 0.75	− 1.75 * 175	1.0	0.8	ARC
X41	F	4	0.5	XT19	XT18	XT12	XT12	1.0	1.0	+ 1.25	0	+ 1.25	0	1.0	1.0	MS
X42	M	34	10	XT31	XT31	XT30	XT35	0.8	1.2	+ 1.00	− 2.50 * 70	+ 0.25	− 0.75 * 155	1.0	1.0	MS
X43	M	39	38	XT18	XT18	XT20	XT15	1.2	1.5	− 0.50	0	0	0	1.5	1.5	NRC III
X44	F	18	8	XT38	XT38	XT42	XT42	0.1	0.1	− 3.75	0	− 4.00	− 0.50 * 125	0.8	0.8	ARC
X45	M	37	20	XT34	XT33	XT35	XT30	0.2	1.0	+ 1.25	− 0.50 * 85	+ 0.75	0	1.0	1.0	MS
X46	M	10	6	XT25	XT27	XT27	XT30	0.12	0.12	− 5.75	− 3.25 * 25	− 4.75	− 3.50 * 165	0.8	0.8	MS

M male, *F* female, *C* childhood, *OD* right eye, *OS* left eye, *ND* not detected, *NA* not applicable, *D* diopter, *SPH* spherical, *CYL* cylindrical, *BCVA* best corrected visual acuity, *MS* monocular suppression, *NRC* normal retinal correspondence, *ARC* abnormal retinal correspondence

Statistical analysis

Statistical analysis was carried out using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA). The Chi-squared tests were applied to compare the complete fibrosis in horizontal rectus muscles among different groups. The Wilcoxon rank sum tests were used to evaluate the correlation between desmin or nestin expression and clinical parameters, including duration of strabismus and degree of deviation.

The duration of strabismus was calculated from age of onset to surgery, and the degree of deviation was determined by the largest value of the deviation in both eyes at distance or vicinity. Differences were considered to be statistically significant at $p < 0.01$.

Results

Clinical findings

The ocular deviations with naked eyes were measured for all of the 46 patients with XT before the surgery by perimeter arc, and the synoptophore was used to obtain the function of binocular vision (Table 1). All of the patients had normal ocular movements. No restriction of movements was revealed in all directions under general anesthesia by the force duction test.

Morphology

The hematoxylin and eosin (HE) staining and Masson's trichrome staining of MR muscle in XT showed almost normal structure of muscle cells (Fig. 1).

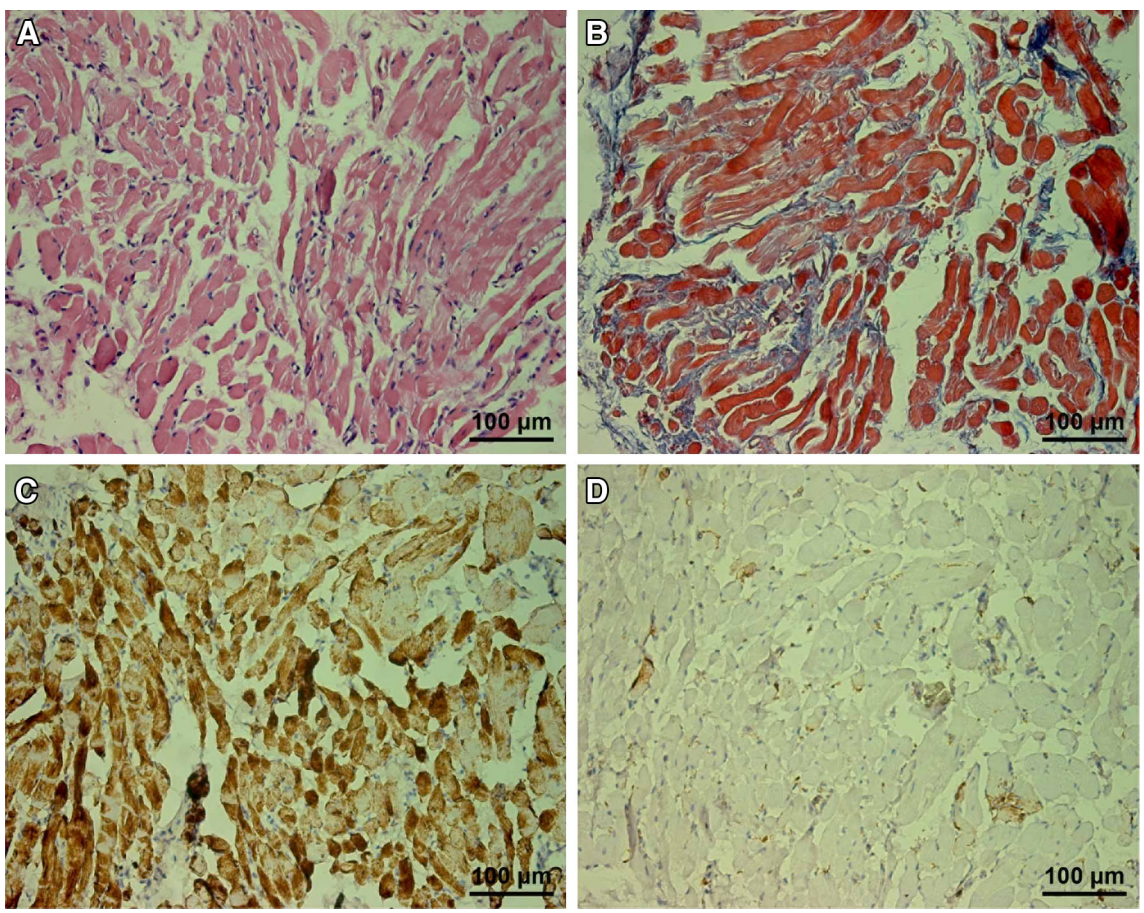

Fig. 1 Hematoxylin and eosin (HE) staining (**a**) and Masson's trichrome staining (**b**) of MR muscle showed almost normal structure of muscle cells in a patient with XT. Immunohistochemical staining of the same sample showed high expression of desmin (**c**), while the expression of nestin was absent (**d**)

Table 2 Immunohistochemical expression of desmin and nestin in medial rectus muscles of patients with concomitant exotropia

Patient	Score of expression (Desmin/Nestin)			
	RLR	RMR	LMR	LLR
X01			2/0	
X02		F/F		
X03		3/1		
X04			5/1	
X05		5/1		
X06			6/1	
X07		6/1		
X08		5/2		
X09		6/1		
X10		F/F		
X11			6/1	
X12		5/1		
X13		6/2		
X14		5/2		
X15			5/1	
X16		5/2		
X17			5/1	
X18			5/1	
X19			4/1	
X20		5/0		
X21			5/2	
X22			6/2	
X23		6/3		
X24			6/2	
X25			5/2	
X26		5/2		
X27		5/4		
X28		6/1		
X29			5/4	
X30			5/3	
X31		5/1		
X32			5/3	
X33		5/1		
X34			5/3	
X35		F/F		
X36		4/2		
X37		6/5		
X38			4/0	F/F
X39		0/0		
X40	F/F	F/F		
X41			F/F	F/F
X42			6/2	F/F
X43			4/1	F/F
X44		4/0		F/F
X45	F/F	0/0		
X46			4/0	F/F

RLR right lateral rectus muscle, *RMR* right medial rectus muscle, *LMR* left medial rectus muscle, *LLR* left lateral rectus muscle, *F* fibrosis completely

Immunohistochemical staining

We found high expression (++/+++) of desmin in the cytoplasm of muscle cells in most (92.7%, 38/41) of samples, while the expression of nestin was low (+) or absent (−) in most (82.9%, 34/41) of samples (Table 2, Fig. 1).

After excluding five MR muscles with complete fibrosis, statistical analysis of the expression of desmin and nestin in cytoplasm of the muscle fibers was processed in 41 MR muscles from patients with XT (Table 2). No significant correlation was observed between desmin or nestin expression and clinical parameters, including duration of strabismus and degree of deviation (Tables 3 and 4).

Discussion

Previous studies had already paid attention to the pathological changes in EOMs in patients with strabismus, showing that fibrous atrophy of EOMs in

Table 3 Correlations between clinical features and the expression of desmin in medial rectus muscles of patients with concomitant exotropia

Clinical features	*n*	Score of desmin expression				*p*
		−	+	++	+++	
Duration						1.0000
≤ 10 years	24	1	1	5	17	
> 10 years	17	1	0	2	14	
Deviation						0.6706
≤ 30°	22	0	1	4	17	
> 30°	19	2	0	3	14	

Table 4 Correlations between clinical features and the expression of nestin in medial rectus muscles of patients with concomitant exotropia

Clinical features	*n*	Score of nestin expression				*p*
		−	+	++	+++	
Duration						1.0000
≤ 10 years	24	6	14	4	0	
> 10 years	17	1	13	2	1	
Deviation						0.6762
≤ 30°	22	3	15	4	0	
> 30°	19	4	12	2	1	

patients with concomitant strabismus was common [11, 12]. However, we detected that MR muscles in XT were almost normal in this study regardless of duration of strabismus and degree of deviation. It may indicate that the pathological change of the EOMs in concomitant strabismus may not be in morphological aspect but in the changes in specific protein composition.

Recent study on distribution of IF proteins in normal EOMs showed that the EOMs differ significantly from the other muscles in the body [8]. According to the previous study in normal LR muscles and superior rectus muscles, desmin was absent or only present in a very low level in a subset of muscle fibers, and nestin was present in a high level in muscle fibers [8]. However, in contrast, we found high expression of desmin in MR muscles of patients with concomitant XT in the present study, and the expression of nestin was low or absent in most of the samples (Table 2).

Desmin, which is the most abundant IF protein in mature skeletal muscles, plays an essential role in maintaining cytoarchitecture, positioning and functioning of organelles, and the intercellular signaling pathway [13–15]. Nestin, which is co-expressed transiently during early development of muscles and downregulated postnatally in skeletal muscles [16–18], is a reliable marker of neural stem cells and is closely correlated with poor prognosis in several tumors [19, 20]. The high expression of desmin and the absence/low levels of nestin in MR muscles of patients with XT indicate that these EOMs had abnormal expression of IF proteins. It has been reported that modifying the surgical dose according to age can improve the success in patients with intermittent exotropia, indicating changes in EOMs with age in horizontal strabismus [21]. But according to the results of the present study, we found no significant correlation between desmin or nestin expression and clinical parameters, including duration of strabismus and degree of deviation (Tables 3 and 4).

Results of this study should be understood within the context of limitations imposed by no normal controls. Because the human EOMs of normal controls can only be obtained at autopsy, we compared the results of this study with the previous study in healthy human rectus muscles. And, we collected the MR muscle samples during the resection process of the strabismic surgery, so the involved samples were closer to the tendon of the MR muscle, and this might have affected the difference in expression of desmin and nestin in the MR muscles.

In conclusion, the present study on changes in IFs in MR muscles in patients with XT showed that the expression of desmin was upregulated in MR muscles in patients with XT, while the expression of nestin was downregulated.

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Compliance with ethical standards

Conflict of interest All 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 and/or national research committee and with the 1964 Helsinki declaration 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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