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Original Article

A Han Chinese infant with spinal muscular atrophy with respiratory distress type 1 (SMARD1) confirmed from a pedigree

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Abstract: Objectives: Spinal muscular atrophy with respiratory distress type 1 (SMARD1) is a rare infantile neurogenic and myogenic disease, leading to misdiagnosis and missed diagnosis easily. SMARD1 is a hereditary disease with mutation of the gene that encodes immunoglobulin μ-binding protein 2 (IGHMBP2). Gene analysis was performed to screen for potential mutations in a Chinese infant with SMARD1. Methods: Medical history, clinical test results and pathology data were collected and analyzed retrospectively. At the same time, related literatures were reviewed systemically. Gene analysis about immunoglobulin μ-binding protein 2 (IGHMBP2) was performed. Results: Muscle weakness of limbs and foot drop developed at 6 weeks of age for the infant, more evident in the distal parts and particularly the lower limbs. At 4 and half months old, respiratory distress appeared suddenly. Chest X-ray displayed the right diaphragm palsy when the infant reached 3 months old. The X-ray of lower limbs showed the volume of calf muscle group was smaller than normal children of 5 months old. Two heterozygous mutations of c.607G>C and c.1418+5G>A were identified at IGHMBP2 by gene analysis. Through pedigree analysis and prediction software identification, the c.607G>C missense mutation may be the pathogenic gene for SMARD1 in this case. Conclusion: IGHMBP2 gene analysis can be useful for early diagnosis of SMARD1. C.607G>C and c.1418+5G>A are two new gene mutations of SMARD1, which improve the gene mutation data to in-depth explore SMARD1 and provide theoretical basis for the subsequent implementation of precision gene therapy.

Keywords: SMADR1, IGHMBP2 mutation, gene analysis

Introduction

Spinal muscular atrophy with respiratory distress type 1 (SMARD1) is an uncommon autosomal recessive motor neuron disorder, one of the variant types of infantile spinal muscular atrophy (SMA). So far, about 60 cases have been reported world wild. SMARD1 is a relatively common autosomal recessive disease among Caucasians but rare in Han Chinese. By now, only 1 case has been reported in China. SMARD1 is usually characterized by progressive distal muscular weakness (particularly at lower limb muscles) symmetrically. Foot deformities, peripheral sensory neuropathy, autonomic nerve dysfunction and sudden respiratory failure are among those characterizations of SMARD1 due to irreversible diaphragmatic paralysis, which requires urgent intubation. Additionally, SMARD1 can be characterized by fatty finger pads over the proximal phalanges which results from the replacement of atrophic muscle by adipose tissue [1].

SMARD1 is a hereditary disease with mutation of the gene that encodes immunoglobulin μ-binding protein 2 (IGHMBP2) [2] that locates at chromosome 11q13. Several reports have revealed that IGHMBP2 regulates cell death of motor neurons [2-5]. IGHMBP2 is a ubiquitously expressed ATPase/helicase within the SF1 superfamily [6, 7], which is reported to be associated with ribosomes and has been functionally linked to mRNA translation [8]. However, how IGHMGP2 mutation causes motorneuron dysfunction remains unclear.

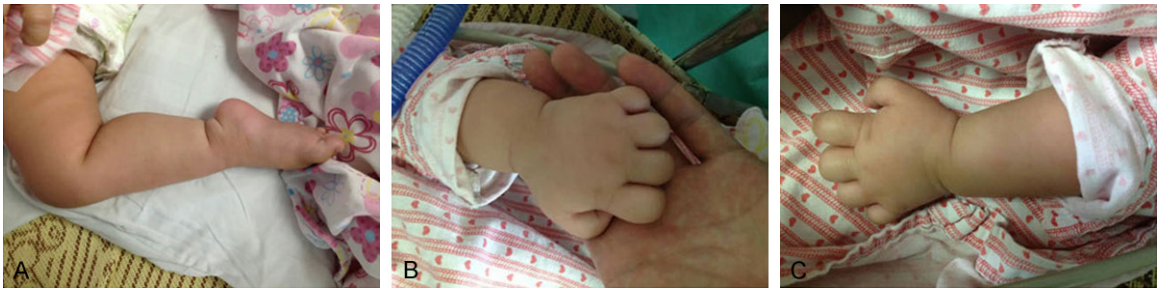

Figure 1. The lowed limbs and fingers of the infant. (A) Lower limb muscle weakness and foot drop, (B, C) finger contracture. All above demonstrated the characteristics of SMARD1.

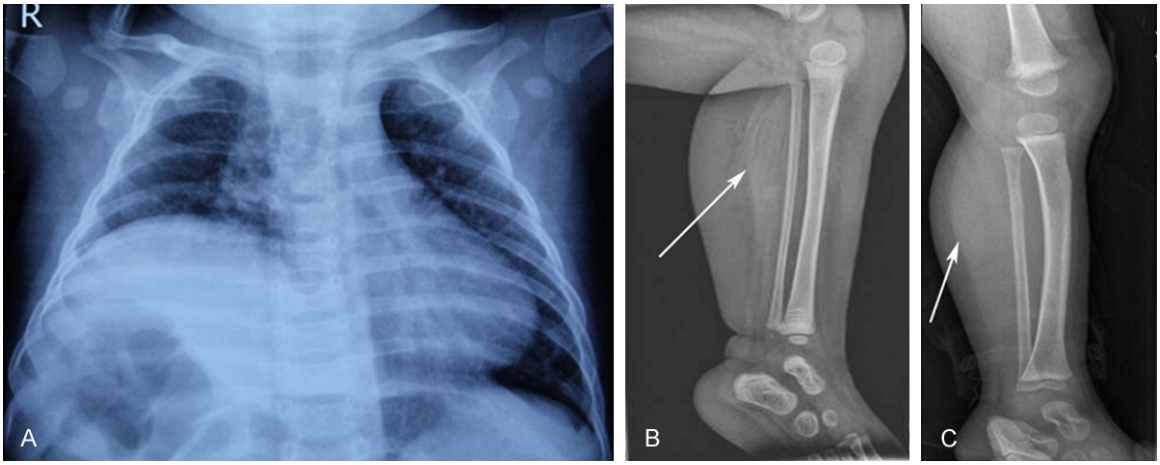

Figure 2. The X-ray of chest and lower limb. A. Chest radiograph showed the elevation of the right diaphragm. B. An X-ray of the low limb displayed the volume of calf muscle group was obviously smaller than normal, demonstrating atrophy. C. The normal X-ray of the left lower limb of the boy at the same age (1 year old).

Untilnow, most of pedigree analyses are ranged from patients to parents. Little data is available about pedigree analyses ranging from patients to their grandparents. Here, the integrity pedigree of SMARD1 was analyzed and two new mutations were identified, providing new method to screen the potential mutation for early diagnosis.

Case presentation

This female infant was born at term by spontaneous vertex delivery. Her parents were healthy non-consanguineous Han Chinese. The antenatal history was unremarkable. No decrease or increased in fetal movement was reported. Birth weight was 3 kg. Family history was negative for neuromuscular disorders or sudden death syndrome. Feeble cry and weak suction were noticed two days after the birth. Weakness of distal lowerlimb muscles and feet drop developed at 6 weeks of age (**Figure 1A**). She was admitted at local hospital and SMA was initially speculated. Prednisone and immunoglobulin were applied and no treatment responses were observed. At the age of 4 and half a months, the child developed sudden respiratory distress which required intubation and mechanical ventilation. Finger contracture occurred at aged of 5 months (**Figure 1B**, **1C**). Laboratory test results showed mildly elevated creatinine kinase (CK) and lactic dehydrogenase (LDH) in the blood. Blood test (thyroid function, lactate, ammonia, amino and organic acids), urine amino and organic acids, the activity of α-glucosidase (the enzyme of glycogen storage disease) as well as brain and spinal cord magnetic resonance imaging all displayed normal. Chest X-ray revealed the right diaphragmatic palsy at 3 months old (**Figure 2A**). Chromosome microarray analysis showed a 400 Kb and a 462 Kb microsatellite repeats detected at 13q14.11 and 14q32.33 respectively. SMA was considered by gastrocnemius biopsy (**Figure 3**). How-

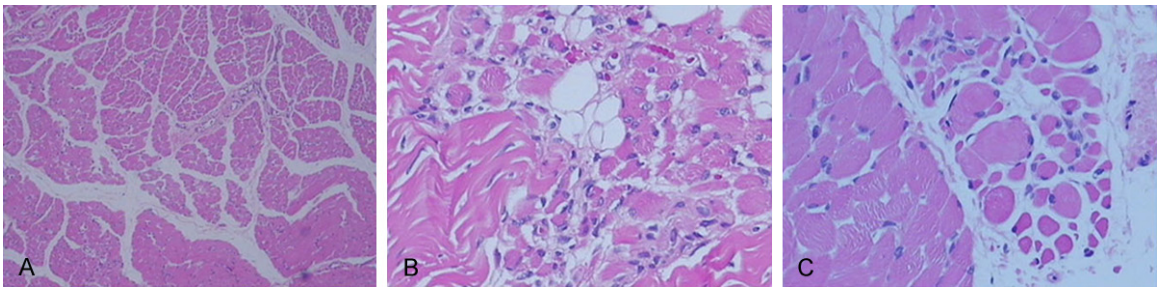

Figure 3. Histopathological alterations of gastrocnemius. Hematoxylin-eosin stain; original magnification ×100 (A), ×200 (B). ×400 (C). Muscle fibers vary in size and atrophy in groups and glassy degeneration was occasionally observed, demonstrating neurogenic lesion.

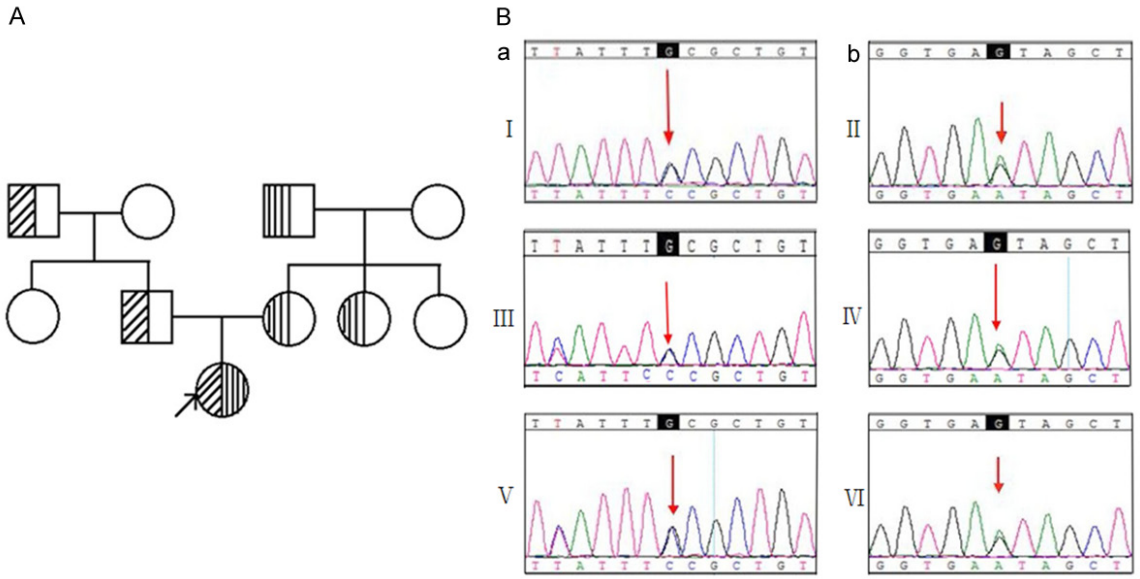

Figure 4. Sequencing of IGHMBP2. A. Pedigrees legend and results for the molecular analysis of the IGHMBP2 gene in a Chinese SMARD1 infant and her family: blank square - healthy male, blank circle-healthy female, square with bias- the healthy male carrier of the mutation c.607G>C, square with long string - the male healthy carrier of the mutation c.1418+5G>A, circle with long string - the female healthy carrier of the mutation c.1418+5G>A, Arrow, proband. B. I-grandfather, II-maternal grandfather, III-father, IV-mother, V and VI-infant. a. Missense mutation c.607G>C; b. Splice region mutation c.1418+5G>A.

ever, gene analysis of SMN1 deletion was negative, which did not support the diagnosis of SMA. An X-ray of the lower limbs displayed the volume of calf muscle group was smaller than normal (**Figure 2B**). Gene analysis results of the infant and the parents showed that the child carried the missense mutation c.607G>C and splice region mutation c.1418+5G>A, both located at IGHMBP2. The father was a carrier of the mutation c.607G>C, while the mother was a carrier of the mutation c.1418+5G>A. However, these two mutations were not common sites associated with SMARD1 reported by previous studies. For further confirmation, gene analysis of second-degree relatives was carried out. The result showed that the mutation of c.607G>C was passed to her father from her grandfather, while the mutation c.1418+5G>A was passed to her mother from her maternal grandfather. Unluckily, these two mutations were both passed onto the infant (**Figure 4**).

Discussion

SMARD1 is a rare hereditary disease with motor neuron disorder. Characteristic clinical features were demonstrated (**Figure 5**). SMADR1 can be categorized into infantile and juve-

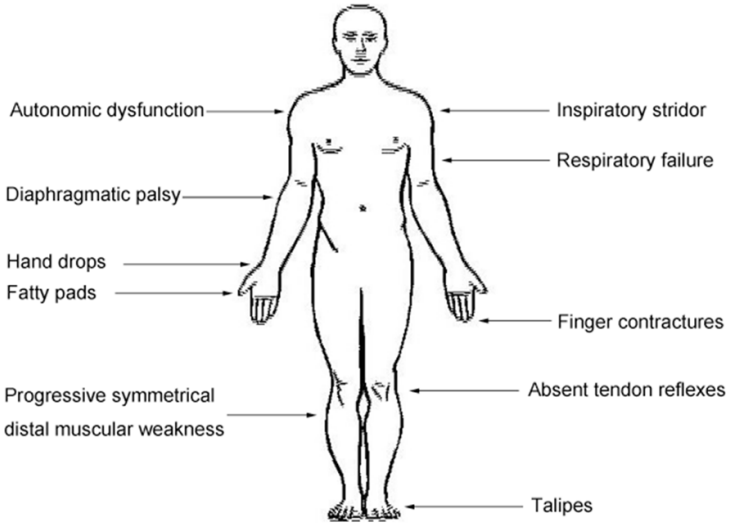

Figure 5. The typical clinical symptoms of SMARD1.

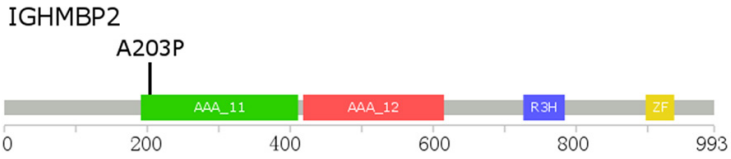

Figure 6. Domain structure of the IGHMBP2 protein. Amino acids p.203Ala is affected by the missense c.607G>C mutation (from A to P).

nile two types. SMARD1 involves infants mostly [9]. The severity of infantile SMARD1 ranges from bedridden to non-ambulant wheelchair bound children [10]. Apart from the classic infantile SMARD1, only a few late onset or mild presentations have been reported [6, 11]. Generally, juvenile SMARD1 is milder compared to infantile SMARD1 in clinical course. It is not common in juvenile SMARD1 with a late onset of respiratory distress and weakness of "foot drop" [10, 12].

In this case, chest X-ray at the age of 3 months demonstrated the elevation of the right diaphragm, three intercostal spaces upper than the left, suggesting a sign of diaphragmatic paralysis. Combining the weak cry, weakness suction, distal lowerlimbs weakness, diaphragm paralysis, fat pads fingers and calf muscle atrophy, SMARD1 was highly suspicious. To further confirm the diagnosis, we did a gastrocnemius biopsy. Pathology result confirmed the diagnosis of SMARD1 in this case.

SMARD1 is caused by loss of function mutations of the IGHMBP2 gene. IGHMBP2 is a component of the translational machinery and that these components can be manipulated genetically to suppress motor neuron degeneration [13]. IGHMBP2 gene consists of 15 exons and has 4 domains: an ATPase domain, a single-stranded nucleic acid-binding R3H domain, a DEXDc domain and an AN1-type zinc finger motif [13, 14]. Previous observations suggest that IGHMBP2 is a multifunctional protein that affects various cellular functions, including transcription, recombination, replication, RNA editing in nuclear and translation intocytoplasm [15-17]. Although the specific role of IGHMBP2 is still not clear, it has been indicated that IGHMBP2 mutations reduces RNA dependent ATPase activity. Juvenile SMARD1 is milder compared to infantile SMARD1, which may be correlated with higher residual levels and enzymatic activity of IGHMBP2 protein [10, 12]. While the mutation of c.1478C>T (p.T493I) decreases stable state of IGHMBP2 [10]. Data shows that a compromised activity, reduced steady state levels of IGHMBP2 or reduced capacity to unwind RNA might reveal the molecular basis for SMARD1. Moreover, further research demonstrated that the duplicated GGAA motifs are essential for IGHMBP2 [18].

To March 2013, a locus-specific database (Leiden database) lists had published variants of the IGHMBP2 gene, providing useful information for genetic counseling (www.dmd.nl). More than 170 IGHMBP2 gene mutations of SMARD1 have been detected. Verified IGHMBP2 gene mutations contain heterozygous and homozygous. Mutations are distributed along all the 15 exons of IGHMBP2 gene. It is more frequent in exons 10 and 12. A predominance of c.1730T>C (p.L577P) missense mutations in exon 12 and the nonsense c.1488C>A (p.C496X) and missense c.1478C>T (p.Thr493Ile) mutations in exon 10 were identified [19]. In the present study, the c.607G>C (p.A203P) missense mutation is located in the fifth exon of IGHMBP2 gene (**Figure 6**), while another c.1418+5G>A

Table 1. Clinical and genetic data of some SMARD1 patients

Ref	Patients NO.	Age at onset of muscular weakness (mo)	Age at onset of respiratory distress (mo)	Diaphragmatic involvement (mo)	Age at death (mo)	IGHMBP2 mutations	PolyPhen-2 score	Type of mutations
Mellins 1974	2	1	1-2	Diaphragmatic paralysis reported for the first time	-	-	-	-
Grohmann 2001	9	1-2	1-2	Eventration of diaphragm	2-2.5	c.-136_-135insCGCCATCTTCCCGC P.(?) + c.2611+1G>T, c.2611+1G>TP.(?)	-	-
Grohmann 2003; Diers 2005	29	1-4	1-6	Diaphragmatic paralysis	-	c.1738G>A p.(Val580Ile)-Homozygous c.2922T>G p.(Asp974Glu) c.114del p.(Glu39Serfs*10) c.121C>T p.(Gln41*) c.138T>A p.(Cys46*) c.388C>T p.(Arg130*)	1.000	2 missense mutations
Pitt 2003	13	0.5-2	<3	Early onset of respiratory failure	3-9	c.127C>T p.(Arg43*)	-	-
Appleton 2004	1	0.5	1.5	Eventration of right hemidiaphragm	-	c.2368C>T p.(Arg790*)	-	-
Guenther 2007	6	1-5	2.5-6	Eventration of diaphragm	-	c.50T>C p.(Leu17Pro) c.1488C>A p.(Cys496*)	0.998	
Messina 2012	1	4	No severe sign	A right diaphragmatic palsy	Alive	c.1G>T (-) IVS 13	-	
Eckart 2015	11	2-8 y	2-9 y	Diaphragmatic paralysis	Some survival	c.2611+1G.T/c.2611+1G.T (-) c.1738G.A/c.1738G.A (p.V580I/p.V580I) c.707T.G/c.721T.C (p.L236X/p.C241R) c.1693G.A/c.1730T.C (p.D565N/p.L577P) c.1707C.T/c.1826C.A (p.R570X/p.A609E) c.1082T.C/c.1730T.C (p.L361P/p.L577P) c.1478T.C/c.2363C.T (p.T493I/p.R788X)	- 0.90 0.92 0.93 1.00 1.00 0.93	
Hamilton 2015	1	16 (No severe sign)	16 (No severe sign)	Eventration of the right hemi-diaphragm	Alive	c.1478C>T (p.Thr493Ile) c.464T>A (p.Leu155Gln)	0.96	

*Represents unknown proteins.

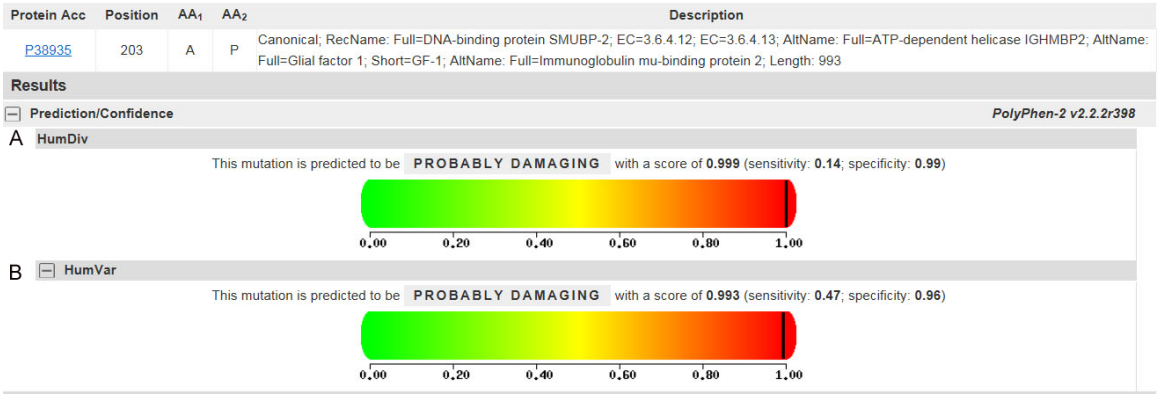

Figure 7. The missense c.607G>C mutation 607G>C is inferred as the causative gene. A. The mutation is predicted to be probably damaging with a score of 0.999 (sensitivity: 0.14; specificity: 0.99) by HumDiv. B. The mutation is predicted to be feasible harmful factor with a score of 0.993 (sensitivity: 0.47; specificity: 0.96) by HumVar.

splice region mutation situated in the ninth of intron. Up to now, only a mutation has been detected in a Chinese girl with an age of 4 years and 10 months [20]. The mutation c.607G>C (p.A203P) leading to no adenosine triphosphatase and ribonucleic acid helicase activity is based on IGHMBP2 protein.

In 2001, the relation of IGHMBP2 gene mutations to SMARD1 phenotype was first discovered in 6 different SMARD1 families [21]. A recent study indicated that patients with homozygous mutations showed a more severe phenotype than patients with compound heterozygosity [22]. However, whether there are correlations between the types of mutation and the phenotypes is still not clear. In addition, no complete clinical differences have been observed among patients carrying different types of mutations.

Parts of reported mutations detected in IGHMBP2 gene (**Table 1**), but not all these mutations affect protein structure and/or function. It is necessary to apply PolyPhen-2 software to investigate the missense mutations. This software predicts possible impacts of amino acid substitution in the structure and function using straightforward physical and evolutionary comparative issues. Score 0 is considered as probably benign, while score 1 as possibly damaging. Therefore, in this infant, the two scores of 0.999 and 0.993 were both close to 1 (**Figure 7**), suggesting the c.607G>C missense mutation might impair protein structure and/or function and potentially initiates the disease. Most children presented suddenly symmetry myasthenia of limbs from 1 month to 5 months old. And respiratory distress presents within 6 months, always along with diaphragmatic paralysis. The majority of patients die within the first year of life and only few survived longer with no severe signs (**Table 1**). These observations are in agreement with preceding literatures [23-25].

So far, the natural history of SMARD1 as well as its exact prevalence is not known yet [26]. Currently, there are no effective therapeutic strategies for the disease. To further recognize and manage this uncommon disorder, preclinical research has been putting a great effort recently. In addition, various treatments such as molecular, gene and stem cell therapy have been developed [27]. Exciting, gene therapy seemed to be more efficient when administered at pre-symptomatic stages in some experimental patients [28, 29]. While stem cell transplantation could play a positive role in symptomatic patients by multiple mechanisms, for example neuroprotection and cell replacement [19]. However, further basic and translational researches are needed to further understand and improve the outcome of the disease.

Taken together, we identified C.607G>C and c.1418+5G>A as two new gene mutations of SMARD1 in this study, which improve the gene mutation data to explore SMARD1 and provide theoretical basis for the subsequent implementation of precision gene therapy. IGHMBP2 gene analysis can be used for early diagnosis of SMARD1.

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Disclosure of conflict of interest

None.

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