

Primary Focal Hyperhidrosis in a New Family Not Linked to Known Loci

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Key Words

Primary focal hyperhidrosis • Genetics • Linkage analysis • Autonomic nervous system • Cardiovascular reflex test • Skin biopsy

Abstract

Background: Primary focal hyperhidrosis (PFH) is a disabling disorder. A first locus has been mapped in families with only palmar involvement, raising the question whether other unknown genes are responsible for more diffuse phenotypes.

Objective: We investigated a PFH family with a new phenotype, providing evidence that PFH is a clinically and genetically heterogeneous condition. **Methods:** Family members were examined by autonomic tests, skin biopsy and genetic analysis, and followed up for 4 years. **Results:** Age at onset was early, involving the axillae, palms and soles. Affected members had dysautonomic features also at onset. Cardiovascular dysautonomia was present in affected and unaffected members. Skin biopsy revealed impairment of intraepidermal nerve fibers and reduced innervation of sweat glands. There was no linkage to PFH and aquaporin-5 loci.

Conclusion: This pedigree may serve as a basis for identifying a novel unmapped gene. Skin biopsy and cardiovascular autonomic tests provide important additions to the characterization of PFH.

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Introduction

Primary focal hyperhidrosis (PFH) is a disorder of excessive sweating that occurs in the axillae, palms, soles and craniofacial region in amounts greater than needed for thermal regulation [1]. This disorder is a serious problem that can have a severe impact on daily functioning as well as cause social phobia, anxiety and depression [2, 3]. There is also an increased risk of developing cutaneous infections, most likely as the result of increased skin moisture [4].

The pathophysiology of PFH is poorly understood. PFH is believed to stem from overactivity of the cholinergic sympathetic innervation to the normal eccrine sweat glands in the affected area leading to excessive sweating [5, 6]. Interestingly, more recent studies have shown cardiac autonomic dysfunction in patients with PFH, suggesting that the neurogenic overactivity is potentially part of a more complex spectrum of autonomic dysfunction, involving both the sympathetic and parasympathetic pathways [7–9].

According to current diagnostic criteria, a definite diagnosis of PFH is established when a subject has focal, visible, excessive sweating of at least 6 months duration in the axillae, palms, soles or craniofacial region [1]. Age of onset <25 years and positive family history are among

the diagnostic descriptors. Unfortunately, these criteria do not allow identification of clinical PFH subtypes due to the clinical and genetic heterogeneity of this condition. In familial cases, the pattern of inheritance suggests an autosomal dominant mode of transmission with variable penetrance [10, 11], and a locus for palmar PFH has recently been mapped on chromosome 14q in families with autosomal dominant inheritance [12]. These patients had only palmar involvement, raising the question whether other yet unknown genes may be responsible for more diffuse phenotypes.

We report here a large PFH family with autosomal dominant transmission and involvement of the palms, axillae and soles. This family does not link to the 14q locus. We also tested the gene encoding aquaporin-5 (AQP5), located in 12q13, because of its expression at the plasma membrane level of sweat glands and its essential role for secretion. Nejsum et al. [13] demonstrated that AQP5-deficient mice show a significant decrease in pilocarpine-induced sweat responses when compared to wild-type animals, highlighting a relevant role of AQP5 in sweat production. Based on this evidence, we selected AQP5 as a potentially interesting candidate for hyperhidrosis in this family.

Patients and Methods

Genealogy and Clinical Evaluation

The proband came to our attention in 2004. Since then, information on the pedigree of her family has been collected by means of historical information, family records and medical chart review, along with interviews of family members. All subjects participating in the study were requested to sign an informed consent. Subject history addressed a detailed list of questions regarding hyperhidrosis: pattern of sweating (duration of symptoms, frequency, volume, areas of involvement, symmetry, specific triggers, presence of sweating during sleep); age at symptom onset; impact of sweating on daily activities and quality of life; family history; associated symptoms that suggest secondary causes of hyperhidrosis. Subjects were requested to check out a complete list of autonomic symptoms assessing the orthostatic, secretomotor, genitourinary, gastrointestinal, pupillomotor and vasomotor systems. All participants underwent physical examinations that were also indicated by any suggestion of secondary hyperhidrosis. The Hyperhidrosis Disease Severity Scale [14], a 4-part questionnaire that provides a quantitative measure of disease severity based on how it affects daily activities, was chosen to measure disease severity in each family member. All family members were reevaluated after 4 years in 2008.

In each subject, the diagnosis of PFH was made based on established criteria [1]. To further disclose secondary causes of excessive sweating, the proband underwent a full laboratory blood test battery, a complete panel of serum autoantibodies, blood tests

to detect inflammation and women's hormone profile, a dosage of plasma and urinary catecholamine, and a complete electromyography and nerve conduction studies.

Genetic Investigations

For linkage analysis, we selected the only known PFH locus on chromosome 14q [12] and a potential candidate gene for hyperhidrosis, the AQP5 gene on chromosome 12 [13]. Blood samples were taken from consenting family members and spouses for genetic analysis. Only patients matching diagnostic criteria for PFH [1] were considered affected for linkage purposes. Linkage analysis was performed by genotyping highly polymorphic microsatellite markers spanning both the PFH and the AQP5 loci. In particular, 5 (D14S261, D14S283, D14S275, D14S70, D14S288) and 2 (D12S85 and D12S368) markers were PCR amplified using fluorescently labeled primers and run on a 3130 xl capillary sequencer (Applied Biosystems). Haplotypes were constructed based on the minimal number of recombinants. Pairwise logarithm of odds scores were calculated using the MLINK option of the FASTLINK software package, assuming autosomal dominant inheritance, a disease allele frequency of 0.001 and incomplete penetrance (0.3–0.8%). For linkage purposes, the 3 clinically unaffected family members (II:1, IV:2 and IV:3) were considered as cases with 'unknown disease status'.

Cardiovascular Autonomic Testing

All the available family members (including affected and not affected subjects) underwent cardiovascular reflex tests directed to evaluate the cardiovascular autonomic function. Drugs that could modify autonomic responses were withdrawn before the assessment. The standard battery consisted of 8 noninvasive tests assessing the cardiovascular parasympathetic and sympathetic function: tests of parasympathetic cardiovascular function included heart rate responses to deep breathing, hyperventilation and the Valsalva ratio; tests of sympathetic adrenergic vascular function included blood pressure responses to standing, head-up tilt, mental arithmetic, cold pressor, isometric hand grip and the Valsalva overshoot. Autonomic testing was performed using standard clinical methodology as previously described [15].

Skin Biopsy

Skin biopsies were performed bilaterally at the glutei and the distal site of the leg (10 cm above the lateral malleolus) on the proband (III:1) using a 3-mm disposable punch after topical anesthesia with lidocaine. Tissue was processed using the free-floating technique previously described [16] by immunostaining with polyclonal antiprotein gene product 9.5 antibodies (Ultraclone, Wallow, UK). The density of intraepidermal nerve fibers (IENF) was quantified in 3 nonconsecutive sections [17]. Briefly, all IENF crossing the dermal-epidermal junction, excluding fragments and secondary branching, were counted and their number was divided by the length of the epidermis to obtain the linear IENF density (IENF/mm). IENF density at the distal leg was compared to available age and gender-corrected normative values [18]. Sweat gland innervation was examined by a semi-quantitative approach.

Table 1. Demographic and clinical data of the 6 definitely affected subjects

Subject	Age at onset, years	Disease duration, years	Hyperhidrosis disease severity scale score	Topography of hyperhidrosis		Other autonomic symptoms	
				onset	last visit	onset	last visit
II:3 (M)	12	56	2	Axillae, palms, soles	Axillae, palms, soles	Intermittent toes blanching and coldness	Intermittent toes blanching and coldness
III:2 (F), proband	6	42	4	Axillae, palms, soles	Axillae, palms, soles, face, gluteal folds	Intermittent digital blanching and coldness, hypersalivation	Intermittent digital blanching and coldness, hypersalivation, orthostatic symptoms
III:3 (M)	4	40	3	Axillae, palms, soles	Axillae, palms, soles	Intermittent digital blanching and coldness	Intermittent digital blanching and coldness, hypersalivation, orthostatic symptoms
III:5 (M)	6	28	4	Axillae, palms, soles	Axillae, palms, soles	Intermittent toes blanching and coldness	Intermittent toes blanching and coldness, hypersalivation, orthostatic symptoms
IV:1 (M)	5	16	3		Axillae, palms	Intermittent digital blanching and coldness, hypersalivation, orthostatic symptoms	Intermittent digital blanching and coldness, hypersalivation, orthostatic symptoms
IV:4 (M)	5	13	3		Palms, soles	Intermittent finger blanching and coldness	Intermittent finger blanching and coldness

Results

Genealogy

The family originates from Northern Italy. It is a 4-generation pedigree with 15 individuals, where PFH has an autosomal dominant mode of transmission (fig. 1). We were able to investigate 9 family members. A definite diagnosis of PFH was established in 6 family members (II:3, III:2, III:3, III:5, IV:1 and IV:4). One deceased subject (I:2) had symptoms suggestive of PFH based on history. One unaffected subject (II:1) was an obliged carrier based on the autosomal dominant transmission.

Clinical Features

History and clinical data of family members are provided below and summarized in table 1.

Definitely Affected Subjects

Subject III:2

The proband was a 48-year-old woman, who had had her first clinical manifestations at the age of 6, consisting in excessive sweating localized bilaterally at the axillae, palms and soles that caused discomfort and embarrassment with psychological and social impairment mostly because of stained clothes at the armpits and when shak-

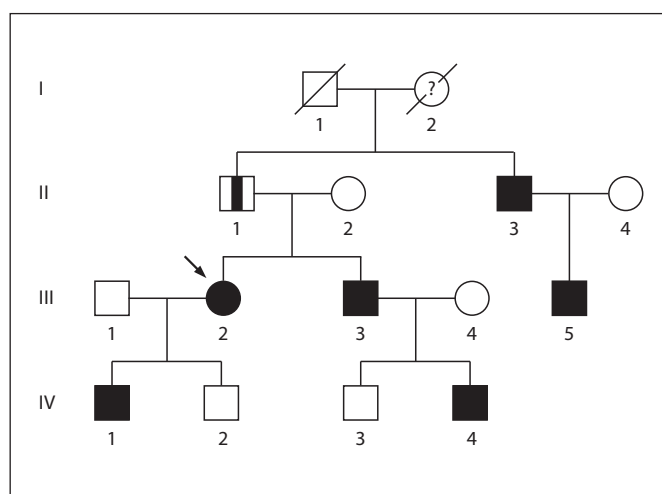


Fig. 1. Pedigree of the family. Filled black symbols indicate examined individuals affected by PFH. The arrow indicates the proband. The thick vertical bar within the symbol indicates an obliged carrier. The question mark within the symbol indicates an individual affected by history (not examined). The diagonal lines indicate deceased members. Empty symbols indicate unaffected relatives.

Table 2. Summary of cardiovascular autonomic responses in the 6 definitely affected subjects and in 1 unaffected subject

Cardiovascular reflex testing	Definitely affected subjects						Unaffected
	II:3	III:2	III:3	III:5	IV:1	IV:4	IV:3
Tests of sympathetic adrenergic vascular function							
Standing	↓	↔	↓	↔	↑	↔	↔
Head-up tilt	↔	↑	↓	↔	↑	↔	↔
Isometric hand grip	↔	↑	↔	↔	↑	↔	↑
Cold pressor	↔	↔	↓	↓	↓	↓	↓
Mental arithmetic	↑	↑	↓	↓	↓	↓	↔
Valsalva overshoot	NA	↑	↔	↔	↔	↔	↔
Tests of parasympathetic cardiovagal function							
Valsalva ratio	NA	↔	↔	↓	↔	↔	↔
Deep breathing	↓	↔	↔	↓	↔	↔	↔
Hyperventilation	↔	↑	↑	↔	↑	↑	↑

NA = Not applicable; ↔ = normal response; ↑ = enhanced response; ↓ = insufficient response.

ing hands and writing. No sweating disturbances were present during sleep; sweat secretion was excessive during waking hours and enhanced by a warm environment and physical-emotional stress; she had skin maceration of the palms during summertime. Since adulthood she had suffered from anxiety and was treated with benzodiazepines because of the distress caused by hyperhidrosis. At the age of 43 she started complaining of excessive sweating on the face and in the gluteal folds triggered by even mild physical exercise. In the past she had been treated with botulinum toxin applied to the axillae and hands. This was effective but poorly tolerated by the patient; more recently, she had successfully used tap water iontophoresis to control palmar hyperhidrosis.

Her autonomic symptoms progressed until the age of 48 and afterwards sweat production improved, reducing its impact on daily life. In childhood she had additionally suffered from intermittent blanching and coldness of the fingers and toes precipitated by exposure to cold; pain and paresthesias were frequent during the attacks and the rewarming of the hands and feet restored normal color and sensation. She also complained of hypersalivation since childhood, with sialorrhea at night; she recalled the dentist pointing out she had excessive salivation. In her thirties she developed symptoms of orthostatic dizziness without experiencing syncope.

Her neurological and general examinations were normal. General laboratory blood testing, serum autoantibodies, plasma and urinary catecholamines, follicle-stimulating hormone and estradiol levels, and electromy-

ography/nerve conduction studies were also found to be normal. Cardiovascular autonomic tests showed a pattern of sympathetic and parasympathetic dysfunction (table 2). Skin biopsy revealed a decrease in IENF density in both glutei (3.7/mm) and distal leg (3.8/mm and 3.4/mm; normal value 5.7/mm), and reduced innervation of sweat glands (fig. 2).

Subject II:3

This 68-year-old man developed bilateral axillary, plantar and palmar hyperhidrosis at the age of 12 years. Excessive sweating was enhanced by emotional and physical stress and it did not occur during sleep. Hyperhidrosis was mildly disabling and sometimes impacting on daily life. At the age of 12 he also complained of cold sensitivity of the toes, which became white and painful when exposed to cold. His autonomic symptoms decreased after he was 63.

Neurological and general examinations were otherwise normal. He only received treatment for hypertension. Cardiovascular autonomic tests showed sympathetic and parasympathetic dysfunction (table 2).

Subject III:3

The proband's 44-year-old brother experienced onset of hyperhidrosis at the age of 4, with bilateral axillary and palmoplantar excessive sweating. These symptoms were triggered by even mild sportive activity; during a football game he could lose more than 2 kg of body weight because of excessive sweating. He did not present focal sweating

during sleep. Hyperhidrosis caused him social impairment but he never sought a treatment for this problem.

Since childhood, he had also complained of intermittent attacks of blanching and coldness of the fingers and toes with associated pain and paresthesias precipitated by exposure to cold; rewarming of the hands and feet restored normal color and sensation. This disorder worsened over time, and in recent years he had started using an electric blanket to prevent the sensation of cold toes at night. He also complained of hypersalivation during sportive activities and of sialorrhea during sleep starting in his teens. His autonomic symptoms progressed over time, mostly since the age of 24, when he experienced orthostatic dizziness. At 26 years he had 2 episodes of orthostatic syncope.

Neurological and general examinations were otherwise unremarkable. He suffered only from moderate hypercholesterolemia. Cardiovascular autonomic tests showed a pattern of sympathetic and parasympathetic dysfunction (table 2).

Subject III:5

This 34-year-old man had had symptoms of hyperhidrosis since the age of 6, with excessive sweating localized at the axillae, palms and soles. His clothes at the armpits and his socks were almost always wet during waking hours, and sweat secretion was enhanced by warm temperature and sports activities. During nighttime he sweated at the soles in a hot environment. This disorder was distressing and caused him social embarrassment. He adopted strategies to reduce excessive sweating, such as wearing two pairs of socks or shirts, using powerful antiperspirants or wearing breathable shoes.

Since childhood he had also suffered of coldness and blanching of toes, which he described as iced, precipitated by cold exposure. His autonomic symptoms progressed from the age of 13 when he presented occasional symptoms of orthostatic hypotension, with dizziness and blurred vision when standing upright. Starting from 24 years he also had occasional hypersalivation with sialorrhea at night.

Neurological and general examinations were normal, and his clinical history was unremarkable. Cardiovascular autonomic tests showed a pattern of sympathetic and parasympathetic dysfunction (table 2).

Subject IV:1

This was the proband's 21-year-old first-born son. Since the age of 13 he had complained of excessive bilateral axillary and palmar sweating exacerbated by stress.

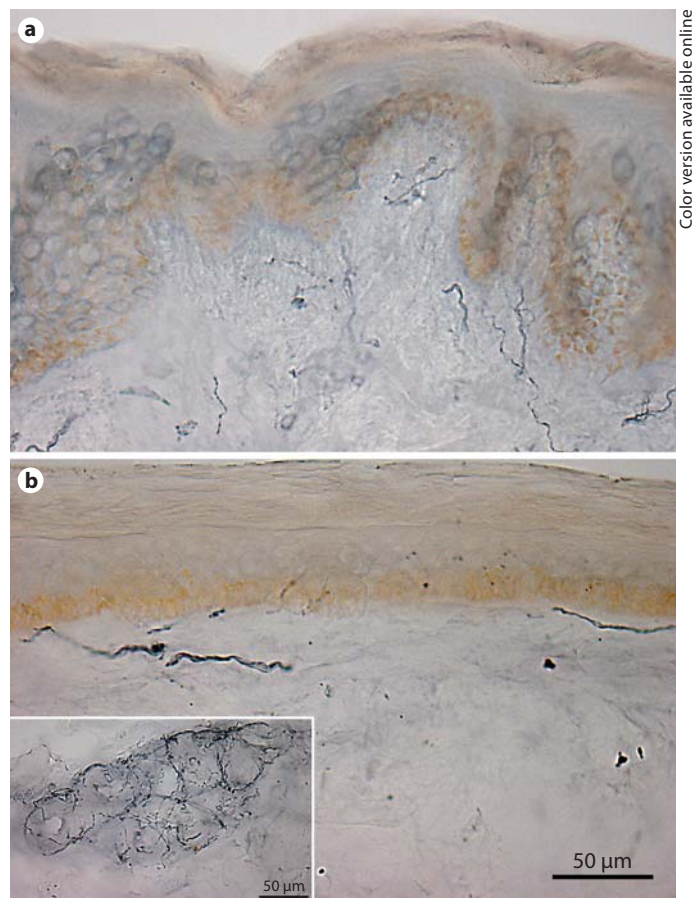


Fig. 2. Skin biopsy sections from the gluteus (a) and the distal leg (b) demonstrating the diffuse loss of epidermal nerve fibers. At both sites, some nerve bundles with degenerative features can be observed in the subpapillary dermis. The inset shows a poorly innervated sweat gland.

This disorder caused him significant distress with intense embarrassment because of sweat dripping from his hands; discomfort and embarrassment were prominent when shaking hands or writing. There were no sweating disturbances during sleep.

Before the onset of hyperhidrosis, since the age of 5, he had suffered from blanching and coldness of fingers and toes, mostly precipitated by stressful situations. Since childhood he had also frequently presented sialorrhea during sleep, and complained of heat intolerance with fatigue and discomfort in hot environments.

His neurological and general examinations were normal, and the clinical history was unremarkable. Cardiovascular autonomic tests showed a pattern of sympathetic and parasympathetic dysfunction (table 2).

Subject IV:4

This 18-year-old man complained of bilateral palmo-plantar excessive sweating since 16 years of age. The disorder caused him discomfort and social embarrassment because of visible wet hands and feet. No sweating disturbances were present during sleep, but during waking hours sweat secretion was excessive and enhanced by warm temperatures and physical-emotive stress. He had to wash his hands very often during the day, mostly in summertime, and he felt uncomfortable when shaking hands and writing because of hyperhidrosis of the palms. The autonomic symptom profile showed coldness and blanching of fingers precipitated by exposure to cold and by stress since the age of 5.

The neurological and general examinations were normal and the medical history unremarkable. Cardiovascular autonomic tests showed altered responses to sympathetic and parasympathetic tests (table 2).

Subjects Affected by History

Subject I:2

She is the proband's paternal grandmother, who was deceased at the time of the study. She had had symptoms suggestive of PFH based on historical grounds. Ever since a child, she used to have excessive bilateral axillary sweating with well-documented social embarrassment; her clothes were stained at the armpits, she had the habit of adding pieces of tissue in her armpits to absorb excessive sweating, and she had to change clothing several times per day.

Clinically Unaffected Subjects

Subjects II:1, IV:2 and IV:3 did not fulfill the clinical criteria for hyperhidrosis, and their neurological examinations were normal. We were able to perform cardiovascular autonomic tests only in subject IV:3 who showed mild autonomic dysfunction involving both the sympathetic and parasympathetic pathways (table 2).

Linkage Analysis

The definitely affected family members did not share a common haplotype for either of the tested loci. In line with this finding, negative logarithm of odds scores at all recombination fractions were generated for all markers (data not shown), excluding linkage of the disease with both the chromosome 14q locus and the *AQP5* gene locus in this family.

Discussion

We report a large Italian family affected by a dominantly inherited PFH not linked to either the 14q or the candidate *AQP5* gene locus. A prolonged observation for 4 years allowed to depict the phenotype and the progression of PFH in this family, which is characterized by: early onset in childhood or early adolescence; hyperhidrosis prevalently involving the axillae, palms and soles but rarely spreading to other somatic areas; nonhyperhidrotic dysautonomic features, such as intermittent digital blanching and coldness, hypersalivation and orthostatic symptoms, which can precede the onset of hyperhidrosis; cardiovascular dysautonomia with sympathetic and parasympathetic dysfunction; slow progression and mild to moderate severity.

This provides further evidence that idiopathic PFH is etiologically heterogeneous. Scanty data are available on the phenotype of 14q-linked PFH [12]: this form involved only the palms in the affected individuals. Other genetically unmapped PFH families with involvement of the palms [10] or of the palms and soles [19] have been reported. Although no laboratory or genetic data are available for these families, their general phenotype resembles the one reported here, suggesting potential genetic commonalities. A phenotype very similar to the one described here has been described in a further PFH family with axillary, palmar and plantar involvement [20]: these two families present remarkably overlapping clinical features, particularly for age at onset and clinical course.

Skin biopsy, showing the loss of IENF in a nonlength fashion, provided the demonstration of the involvement of somatic axons and suggested a primary impairment of small neurons in dorsal root ganglia rather than axonopathy. This subclinical involvement of somatic nerves has been previously reported in patients with Ross syndrome, an autonomic disorder also characterized by the loss of sweat gland innervation not only in anhidrotic, but also in hyperhidrotic skin, as seen in our patient [21]. It is not clear how reduced innervation of sweat glands can produce profuse sweating in hyperhidrotic areas. This could be explained by a compensatory phenomenon or by a denervation hyperactivity phenomenon of the sudomotor function. However, in our patient we performed a qualitative analysis of sweat gland innervation that should be supported by a quantitative measurement [22]. Conversely, in patients with pure autonomic failure, the density of epidermal and sweat gland nerve fibers is preserved, suggesting that anhidrosis is caused by a functional impair-

ment of cholinergic transmission rather than the degeneration of cholinergic nerves [23].

The observation of cardiovascular dysautonomia is in keeping with earlier observations demonstrating that, notwithstanding its name, PFH is a diffuse autonomic nervous system disorder [9]. The nature of autonomic dysfunction, however, remains controversial, as some studies have reported sympathetic overactivity [5, 24], while others argued against it [25] or reported parasympathetic dysfunction instead [7, 8]. In the present family, we report the occurrence of mild alterations of both sympathetic and parasympathetic nerves in all the affected patients and also in 1 clinically unaffected subject. This inconsistency of cardiovascular autonomic findings further supports the clinical heterogeneity of PFH indicating that a full characterization of the broad phenotype is necessary before pooling data from different families for genetic studies. Follow-up of the asymptomatic individual IV:3 will clarify whether the impairment of cardiovascular autonomic tests may precede the onset of hyperhidrosis.

All of the affected individuals have symptoms of Raynaud's phenomenon. This has been reported to occur also in sporadic cases of PFH [26, 27]. Raynaud's phenomenon is not an exclusion criterion for the clinical diagnosis of PFH [1].

We have also excluded linkage to a potential candidate gene for hyperhidrosis, the *AQP5* gene, which is functionally implicated in sweat secretion [13]. This gene has not been investigated in other PFH pedigrees and, at least in this family, it seems not to be involved in the pathogenesis of hyperhidrosis.

In summary, this family with autosomal dominant PFH differs from previously described cases and resembles a previously described family. Skin biopsy and cardiovascular autonomic function tests provide important additions to the characterization of PFH families, particularly as they allow to show the occurrence of subclinical dysautonomia. The present pedigree does not link to mapped PFH loci and may serve as a basis for identifying a novel, yet unmapped PFH gene.

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Disclosure Statement

All authors report no conflict of interest and have nothing to disclose.

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