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FULL-LENGTH ORIGINAL RESEARCH

EFHC1 mutation in Indian juvenile myoclonic epilepsy patient

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Summary

Objective: Juvenile myoclonic epilepsy (JME) is the most common form of idiopathic generalized epilepsies (IGEs) and is genetically heterogeneous. Mutations in *EFHC1* cause JME. Because about 2 million people in India are affected by JME alone, we investigated the prevalence of mutations in the *EFHC1* gene in the Indian population with JME. We studied 63 patients with JME and 80 healthy controls.

Methods: Clinical identification of JME was evaluated using established criteria. Following clinical evaluation of the patients and confirming presence of JME, blood samples were collected from each patient and healthy individual. Subsequently, genomic DNA was extracted from the blood samples. Eleven exons of the *EFHC1* gene were individually amplified by polymerase chain reaction (PCR) for each DNA sample. The PCR products were then purified and sequenced commercially. The identified DNA variants were sequenced at least twice in both the forward and reverse directions and compared with the Exome Aggregation Consortium (ExAC) database.

Results: We found five heterozygous and one homozygous variant. We found three novel coding variants 661C→T, 779 G →A, and 730 C→T, which lead to R221C, R260Q, and R244STOP amino acid substitutions, respectively. The coding variant 475 C→T, resulting in the amino acid substitution R159W, reported earlier as polymorphism, was also identified in both patient and control populations.

Significance: Detection of these three novel variants, excluding R159W, which is considered polymorphism, expands the range of possible mutations in the *EFHC1* gene. The novel variants that we are reporting herein have not been mentioned before as occurring in JME patients of other ethnic population. Therefore, these novel coding variants may be confined to the Indian JME population. Further studies on the mutational spectrum of *EFHC1* in a larger number of Indian JME patients concurrent with their mode of inheritance and underlying functional assays should establish whether *EFHC1* could be a panethnic gene for JME.

KEY WORDS: JME, *EFHC1*, Indian population, Mutation, Polymorphism.

Juvenile myoclonic epilepsy (JME) is the most common form of idiopathic generalized epilepsy (IGE). It accounts for 10–15%[1–3] of all cases of epilepsy. About 2 million people in India are affected by JME. The seizures of JME may begin between late childhood and early adulthood, usually around the time of puberty. JME usually has an onset in adolescence, with the typical age of onset between 12 and 18 years;[4] however, children as young as 6 years and adults as old as 36 years can develop JME. It is characterized by adolescent onset, infrequent absence seizures, awakening myoclonic seizures, and generalized tonic-clonic (GTC) or clonic-tonic-clonic (CTC) seizures. It is also more likely to

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Key Points

- *EFHC1* mutation status in Indian population is reported
- Novel *EFHC1* variants and mutations are identified
- The *EFHC1* coding polymorphism R159W is very rare in the Indian population

occur in people who have family members with generalized epilepsy. Studies investigating the genetic contribution to JME have mainly focused on familial forms with an autosomal dominant inheritance of IGE syndromes.[5,6]

A number of studies have demonstrated that mutations in ion channels as well as in neurotransmitter receptors are associated with JME and include calcium channel subunit *CACNB4*,[5] the γ-aminobutyric acid (GABA) receptor subunit, *GABRA1*,[7] ligand-gated chloride channel for GABA subunit *GABRD*,[8] and the chloride channel *CLCN2*.[9] For each of these genes, mutations have been reported mostly in a single family with JME and most often it is de novo, that is, not observed in the parents. Importantly, these mutations were not observed at all in other family-based JME studies, neither in the same nor in different ethnic populations. Therefore, mutations in ion channel genes can be considered a rare cause of JME.

Analyzing the Mexican cohort samples as described by Bai et al.,[10] Suzuki et al.[6] isolated a new gene, *EFHC1*, which has apoptotic activity and encodes a protein of 640 amino acids with a calcium-binding motif, within the 6p12–11 mapped locus and identified five missense mutations in six independent Mexican JME families out of 31 Mexican JME families. All mutations resulted in single amino acid substitutions. Stogmann et al.[11,12] also sequenced 61 Austrian JME patients and identified three heterozygous missense mutations in the *EFHC1* gene. Annesi et al.[13] studied 27 Italian JME families with 86 affected individuals and reported two heterozygous mutations in *EFHC1* in three unrelated families. Medina et al.[14] further identified five novel mutations in transcripts A and B of the *EFHC1* gene in 4 (9%) of 44 Hispanic patients from Mexico and Honduras and in 2 (3%) of 67 Japanese patients with juvenile myoclonic epilepsy. The latter three studies, therefore, further support *EFHC1* as a JME-causing gene.

However, Pinto et al.[15] reported heterogeneity at the 6p12–11 locus and observed the absence of mutation in the *EFHC1* gene in 112 Dutch JME patients who were previously mapped to the 6p12–p11 locus. Therefore, it seems that multiple genes may be involved in JME, and these may vary between and within ethnicities. One large JME family from Belize that mapped to the 6p12–11 locus carried a common polymorphism of *EFHC1* that cosegregated with JME with higher frequency than in normal individuals. This polymorphism did not have any effect on normal *EFHC1* function, as judged by *EFHC1*-mediated cell death analysis, suggesting the possibility that this family may be linked to *EFHC1* and that another nearby mutation most likely may be responsible for JME in this family.[16]

Subaran et al.[17] questioned the effect of reportedly pathogenic *EFHC1* mutations on JME. The group emphasizes that pathogenicity of *EFHC1* coding variants may depend on genetic backgrounds of the population being studied. Further, the study cautions us about the level of evidence necessary to attribute causation.

Therefore, to establish whether *EFHC1* mutations contribute to JME in populations with different ethnic backgrounds, we screened the *EFHC1* gene for mutations among 63 JME patients originating from 63 independent families in India alongside 80 controls with no history of epilepsy.

Material and Methods

Patient samples

Sixty-three Indian JME patients were analyzed for the present study according to the criteria of the Commission on Classification and Terminology of the International League Against Epilepsy (1989).

Clinical identification of JME was evaluated using the following inclusion criteria: (1) had to have seizure onsets around the adolescent period, between 10 and 21 years of age. All had experienced early morning events, and precipitation of seizures included sleep deprivation. (2) Electroencephalogram (EEG) of the patient often displayed bilateral, diffuse, symmetrical, and synchronous 4- to 4.5-Hz spikes, including polyspikes and wave complexes, either spontaneously or on photic, hyperventilation and in a sleep deprived state. (3) History of seizures with myoclonus and absence seizures. The exclusion criteria for patients included: (1) symptomatic seizures history, (2) presence of progressive myoclonic epilepsy and/or progressive neurological disease in the family, and (3) evidence of complex or partial seizures.

Following clinical evaluation of the patients and confirming presence of JME, blood samples were collected. All subjects provided written informed consent, as required by the institute's ethics committees. It was very difficult to access the blood samples from family members.

DNA sequencing

Peripheral blood samples were collected from each participant, and genomic DNA was extracted using Flexigene DNA Kit (Qiagen). Eleven exons of the *EFHC1* gene were individually amplified by polymerase chain reaction (PCR) using intronic primers. The PCR products were then purified using QIAquick gel extraction kit (Qiagen) and sequenced commercially. The identified variants were sequenced at least twice in both the forward and reverse directions. Screening for mutations in

the *EFHC1* gene was performed in all index patients and controls.

Results

Clinical characteristics

In our cohort of 63 JME patients, 39 (62%) were male and 24 (38%) were female. The average age at onset of epilepsy was 13 years (range 10–21 years). Most cases (73%) were singletons having no positive family history of epilepsy. All the patients met the criteria for classic JME.

Mutation analysis

In 63 JME patients, we identified four heterozygous coding variants, 661C→ T, 779 G → A, 881 G → A, and 730 C→ T, of *EFHC1* that resulted in the amino acid substitutions R221C, R260Q, R294H, and R244STOP, respectively,

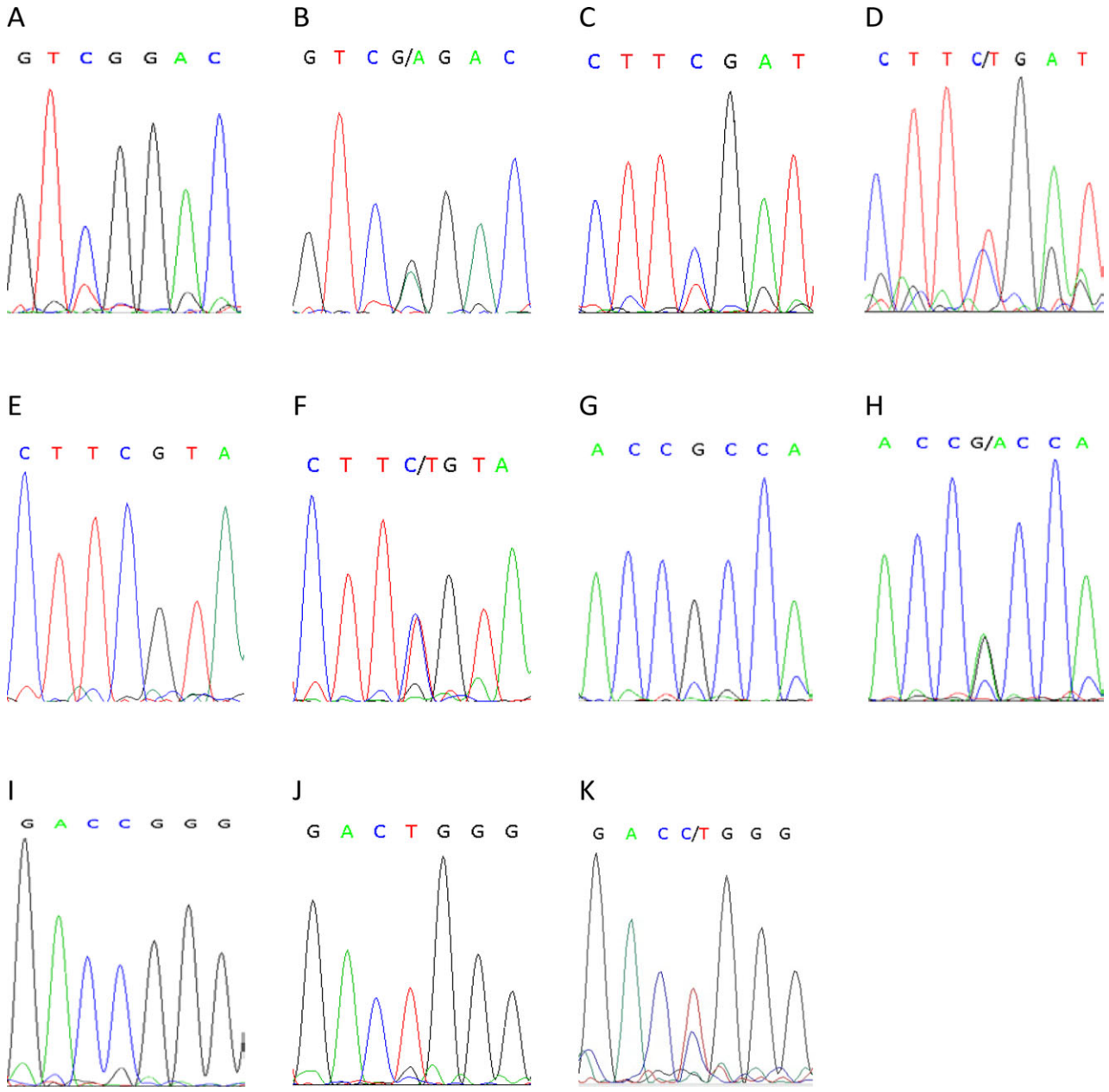

Figure 1.
Chromatogram of the five *EFHC1* variants. (**A**) WT and (**B**) R260Q; (**C**) WT and (**D**) R244STOP; (**E**) WT and (**F**) R221C; (**G**) WT and (**H**) R294H; (**I**) WT and (**J**) & (**K**) R159W (homozygous and heterozygous, respectively)
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and one homozygous and one heterozygous coding variant 475 C→T that resulted in the amino acid substitution R159W (Fig. 1). The identified heterozygous coding variant R221C was found in exon 4, and the other three variants R260Q, R294H, R244STOP were found in exon 5. Homozygous and heterozygous coding variants R159W were found in exon 3 (Fig. 2).

The coding variant R159W is considered a polymorphism and occurred at a frequency of 16% and 14% in Mexican JME families and in the control population, respectively.[6] We detected this variant in 2 JME patients (frequency 3.17%) and in 1 in the control sample (frequency 1.25%). This could be a relatively rare polymorphism in the Indian population.

The coding variants R221C, R260Q, and R244STOP seem to be novel variants because these were never reported in any other ethnic populations. These variants are within the second DM10 domain of the *EFHC1* gene, except R221C, which is very close to the second DM10 domain. The frequencies of the coding variants of R294H, including the novel ones, were 2.32% for each in the Indian JME patient population we studied. None of these variants was found in our Indian control samples. In the Exome Aggregation Consortium (ExAC) database, the frequencies of R221C, R260Q, R294H, and R244STOP variants were found to be 0.18%, 0.057%, 1.92%, and 0.0034%, respectively. As mentioned earlier, because of the lack of samples available from family members, we were unable to infer their *EFHC1* mutational status, which consequently limits our efforts to determine the mode of inheritance. This has prompted us to treat any *EFHC1* variants found in the studied patients as singletons.

R294H mutation was detected in a female patient with JME who had unaffected parents but who had a history of generalized seizures and myoclonus with awakening preponderance in a paternal uncle (Fig. 3A). She had a febrile seizure at the age of 3–4 years, and at the age of 12 years had a myoclonus seizure and proceeded to display generalized seizures at the age of 15 years. She had complaints of headache, frontal throbbing, and phonophobia, but no photophobia, nausea, or vomiting. The R294H variant was reported earlier in two probands of German JME patients who had a subtype of JME developing from childhood absence epilepsy.[18]

A male patient carrying the R159W allele had a mother with generalized seizures and myoclonus with awakening preponderance (Fig. 3B). He had abnormal EEG with generalized spike and wave discharges, but no focal deficits. He had a myoclonic seizure at the age of 16 years that proceeded to form generalized seizures at the age of 18 years. Patients carrying R221C, R260Q, and R244STOP had no affected members in their extended family, including their parents. These patients displayed classical JME features. Therefore, it seems that in these patients JME appears as a singleton.

Discussion

Mutation in *EFHC1* causes JME. We evaluated 63 Indian JME patients for the presence of *EFHC1* mutation. Five heterozygous and one homozygous *EFHC1* variants were found in six independent JME families. Because their mode of inheritance was not ascertained and in some cases there were signs of dominant inheritance, these variants were considered as singletons. Detection of these three novel variants, excluding R159W, which is considered a polymorphism, expands the range of possible mutations in the *EFHC1* gene. It also demonstrates that because mutations in *EFHC1*are found more often in JME patients irrespective of their geographical origin or ethnicity, *EFHC1* may be considered a panethnic JME gene. Because of the limited number of variants we unearthed in the Indian JME patients, we could not establish any correlation between nature of

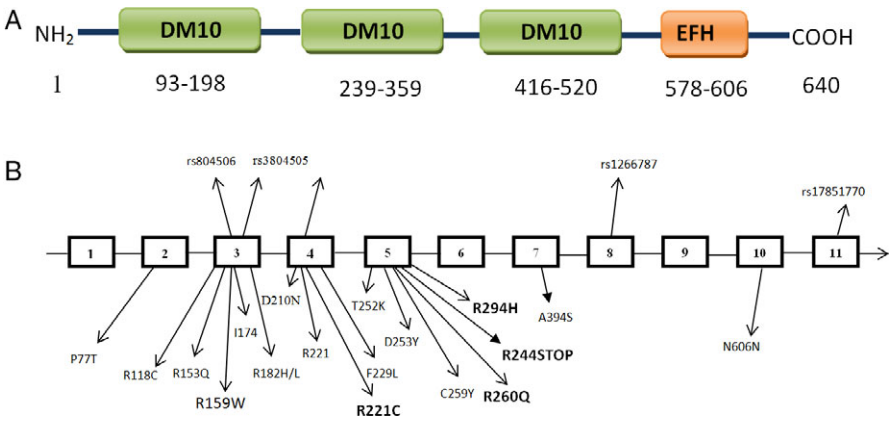

Figure 2.

(**A**) Schematic diagram of EFHC1 protein. (**B**) Genomic organization of the *EFHC1* gene showing the previously reported mutations in different exons, whereas the new mutations observed in the Indian JME patient are shown in bold type.

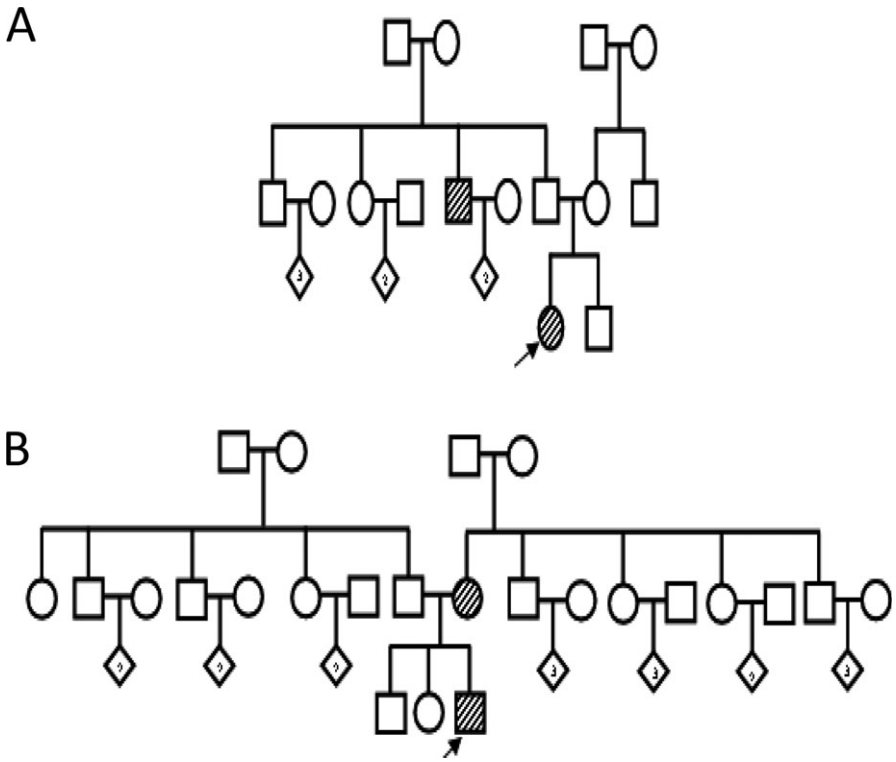

Figure 3.
Pedigrees of the families with JME: (**A**) R294H and (**B**) R159W. Filled symbols indicate affected individuals. Arrows indicate the probands of each family.
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variants and clinical features. However, all affected individuals displayed classic JME symptoms with typical EEG traits of 4- to 4.5-Hz generalized polyspike waves, further supporting the notion that *EFHC1* mutations are associated with classic JME without pyknoleptic absences.[6]

On the possible effects of variants in the functioning of *EFHC1*, polymorphism phenotyping (PolyPhen) analysis predicts that R221C and R294H variants may have damaging effects, whereas R260Q may have negligible effects. The three mutations R221C, R294H, and R260Q received scores of 0.776, 0.998, and 0.087, respectively. Another tool, SIFT, that predicts variation effects on protein function showed scores of 0.01, 0.00, and 0.06 for R221C, R294H, and R260Q, respectively. According to these scores, R221C and R294H are deleterious and R260Q is tolerated. One of the identified *EFHC1* mutations (R244STOP) results in an abrupt stop codon in the middle of the second DM10 domain. Because both of these tools predicted similar consequences for the variants and because their frequency of occurrence in the general population is much lower, according to the ExAC database unphenotyped for epilepsy, including their absence in our control sample comprising 80 people, we assume that these variants may be mutations. Moreover, because these novel variants, except R221C, lie within the DM10 domain of *EFHC1*, it is most likely that functions of *EFHC1* might be compromised.

Interestingly, in a recent article Bailey et al.[19] reanalyzed 54 *EFHC1* variants associated with epilepsy from 17 cohorts and classified 9 variants as pathogenic. This study also included JME mutations (H89R, Y355C, R372W, R436C, N519S, V556L, I619S, and Y631C) reported from India. The majority of these *EFHC1* coding variants originated from Bangalore (R372W, R436C, N519S, V556L, I619S, and Y631C) and may not be pathogenic.[19] Therefore, these are far from the New Delhi *EFHC1* variants that are located primarily between DM10 (1) and DM10 (2) domains. It might be a reflection of the differences in "peopling" effect and ancestral origin of the two different populations of New Delhi (North India) and Bangalore (South India). Subaran et al.[17] recently reported that the pathogenic *EFHC1* P77T-R221H (rs149055334–rs 79761183) JME haplotype is present in both Hispanic and African American controls, including in the public database of unphenotyped West African ancestry populations. We believe that some *EFHC1* mutations may be pathogenic only when introduced into specific genetic backgrounds.

Having said that, however, in the absence of specific biological assays that can monitor the altered function of the protein, the identified novel coding variants of *EFHC1* reported herein cannot be termed mutants with certainty. The same notion may be extended to earlier reported coding variants of *EFHC1*, which are often considered mutants on

the basis of prevalence and cosegregation in JME patients, including modeling on PolyPhen tools.[6,11,13,14,20]

About 263 missense variants for *EFHC1* have been reported in the ExAC database. All the coding variants that have so far been reported to occur in JME patients, including the ones that we have uncovered in Indian JME samples, are found in the ExAC database except N606N, which was reported earlier. However, the novel variants that we are reporting herein have not been mentioned before as occurring in JME patients of other ethnic populations. Therefore, these novel coding variants may be confined to the Indian JME population. Because we identified only four mutations in 63 independent families, it points out that *EFHC1* mutations may not be the only cause of JME in the Indian population. Further studies on the mutational spectrum of *EFHC1* in Indian JME patients concurrent with their mode of inheritance and underlying functional assays should establish whether *EFHC1* could be a panethnic gene for JME. Absence of parental samples in our present study limited our efforts and scientific evaluation of the genetic nature of the variants, that is, whether they are de novo or sporadic. Additionally, we have not analyzed the promoter sequence of *EFHC1* that may also harbor polymorphism that probably affects the transcriptional regulation of *EFHC1*.Therefore, further work is necessary to evaluate and identify the *EFHC1* coding variants status in Indian JME patients.

Conclusion

Mutation in *EFHC1* causes JME. We are one of the first to report studies on the status of *EFHC1* mutation in Indian JME patients. We observed three coding variants (R221C, R260Q, and R244STOP) that were never reported before in any ethnic community. These variants were absent in the Indian control sample. All these novel variants were singletons, and none of their parents have JME. In the absence of a definitive biological process wherein we can assess the mutational effect of *EFHC1*, whether the identified variants of *EFHC1* in Indian JME patients and/or in other ethnic communities are truly mutants remains questionable, and as of now these variants would most likely be risk-allele for JME.

Acknowledgments

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Disclosure

The authors declare no conflicts of interest. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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