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ORIGINAL PAPER

Cystic fibrosis in Afro-Brazilians: *XK* haplotypes analysis supports the European origin of *p.F508del* mutation

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Abstract Cystic fibrosis (CF) is a common autosomal recessive disorder, being the *p.F508del* the most frequent mutation. Also, a nearby restriction fragment length polymorphism (RFLP) named *XK* (*KM19* and *XV2C*) is non-randomly associated with specific CF alleles. Our aim was to analyze the occurrence of the *p.F508del* mutation and *XK* haplotypes in Afro-Brazilians CF patients and controls, since these data is available for the other two main ethnic groups found in Brazil (Euro-Brazilians and Brazilian Amerindians), contributing for the whole comprehension of these haplotypes in the Brazilian population. A total of 103 patients and 54 controls were studied. PCR and PCR-RFLP methodologies were used to identify the presence of the *p.F508del* and the *XK* haplotype in the subjects. The combined data show that 84.2% of *p.F508del* mutation is associated with haplotype B and only 15.8% with haplotype A; no other haplotypes were found to be associated with this mutation. Our data suggest that the occurrence of *p.F508del* mutation and haplotype B in Afro-Brazilian patients occurs probably due to admixture with Euro-descendants. Therefore this mutation and haplotype could be used as a admixture marker.

Keywords Cystic fibrosis · *CFTR* · *XK* haplotypes · *p.F508del* · Afro-Brazilians

Introduction

Cystic fibrosis (CF) is an autosomal recessive disorder with an estimated incidence in Euro-Brazilians of about 1 in 7576 live births, corresponding to a carrier frequency of about 1 in 44 (Raskin et al. 2008). The incidence is lower in Afro-Brazilians being estimated as 1 in 14 085 live births corresponding to a carrier frequency of about 1 in 60 (Raskin et al. 2003). Since the CF gene was mapped to chromosome 7 in 1985, a variety of DNA segments tightly linked to the CF gene have been identified (Estivill et al. 1987a; Knowlton et al. 1985). These segments contain a variety of restriction fragment length polymorphisms

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(RFLPs) that are useful for the analysis of families with a history of CF and for population studies. The *CFTR* gene (Cystic Fibrosis Transmembrane Conductance Regulator) was cloned in 1989 and the most common mutation in this gene is a 3 bp deletion causing the loss of phenylalanine residue at position 508 (*p.F508del; c.1521_1523delCTT; rs113993960*) in its eleventh exon (Kerem et al. 1989; Riordan et al. 1989; Rommens et al. 1989). The frequency of the *p.F508del* mutation in Euro-Brazilians patients is around 48% and in Afro-Brazilians patients is around 12% (Raskin et al. 2003).

Two DNA segments tightly linked to the *CFTR*, called *XV2C* and *KM19*, or simply *XK*, are located 260 kb from the 5′ end of the CF gene and are predicted to have less than a 1% recombination rate with the CF locus (Estivill et al. 1987a, b). Each of the *XK* loci contains a two-allele system and together detects four possible allele combinations or haplotypes per chromosome, named A through D (Beaudet et al. 1989). The distribution of *XK* haplotypes has been determined in large numbers of Euro-descendants CF patients worldwide (Cutting et al. 1989; Estivill et al. 1987a, 1988; Raskin et al. 1997a, b), but in a few CF African patients or Afro-descendants (Cutting et al. 1989; Denter et al. 1992; Lucotte et al. 1989; Martin et al. 1988).

We have previously reported the *p.F508del* mutation and *XK* haplotypes distributions in Euro-Brazilians and Brazilian Amerindians (Raskin et al. 1997a, b, 2007). However, no study about the *XK* haplotypes were performed in Afro-Brazilians, one of the most important ethnicities found in Brazil with high admixture rates with Euro-descendants (in Minas Gerais State it is about 50%, Salzano and Freire-Maia 1967). So, the aim of this study was to analyze the occurrence of the *p.F508del* mutation and *XK* haplotypes in Afro-Brazilians CF patients and controls.

Materials and methods

Subject population

The *p.F508del* mutation and the *XK* haplotypes frequencies were determined in 54 healthy Afro-Brazilians (108 non-CF chromosomes) and in 37 CF patients (74 CF-chromosomes) living in Minas Gerais (MG) State, and in 66 CF patients (132 CF-chromosomes) living in Bahia (BA) State. The Afro-Brazilian patients from both States were grouped in a total of 206 CF-chromosomes. The criteria for diagnosis as cystic fibrosis included pulmonary disease associated with pancreatic insufficiency, meconium ileus or family history of CF, as well as two positive sweat tests. Patients were characterized as Afro-Brazilians if they were not phenotypically "white" and at least one of the parents was an African descendant. The "white" and "non-white" phenotype has been assigned based on known criteria such as skin color, hair color and type, lips and nose shape, as described elsewhere (Azevêdo 1980; Krieger et al. 1965).

Informed consent was provided by all participants and this study was approved by the Research Ethics Committee of PUC-PR (Paraná, Brazil).

DNA analysis

Genomic DNA was obtained from peripheral blood leukocytes following general procedures. The *p.F508del* status of the patients was performed as described elsewhere (Costa et al. 2007). The *XK* alleles were performed by PCR-RFLP (Horn et al. 1990).

Statistical methods

The *p.F508del* mutation, *KM19* and *XV2C* allele frequencies were obtained by direct counting. *XK* haplotypes were designated as described in Table 1 and that ones that were double heterozygous were identified by maximum likelihood analysis. Homogeneity of the populations was verified by analysis with RxC (R by C) program (Miller 1997). Statistical analysis of our data compared to the frequency of the *p.F508del* mutation and *XK* haplotypes in Euro-Brazilians, African and Afro-American patients and controls were performed using the SPSS (*Statistical Package for the Social Sciences*) Statistical 20 software.

Results

p.F508del mutation frequency

No *p.F508del* mutation was found in 108 non-CF chromosomes from Afro-Brazilians living in MG state. We found *p.F508del* mutation in 9 (out of 74) CF chromosomes (12.2%) from Afro-Brazilians CF patients living in MG state and in 10 (out of 132) CF chromosomes (7.6%) from Afro-Brazilians CF patients living in BA state, as shown in Table 1. Besides this difference, there is no statistically significant difference in these two populations, and therefore, the data were pooled ($p = 0.39$).

XK haplotype frequencies

The *KM19* and *XV2C* haplotype distributions found in this study are shown in Table 2. Comparing the data of 132 CF chromosomes from Afro-Brazilians living in BA state and 74 CF chromosomes from Afro-Brazilians living in MG state no statistically significant was found in the XK haplotypes ($p = 0.89$) (Table 2).

Table 1 Distribution of *XK* haplotypes in Afro-Brazilian chromosomes with or without *p.F508del* mutation

Haplotypes[a]	Afro-Brazilians chromosomes									Euro-Brazilians chromosomes[b]			
	Minas Gerais (MG)				Bahia (BA)			MG and BA					
	Controls (108)	Patients total (74)	Patients W 12.2% (9)	Patients WO 87.8% (65)	Patients total 132	Patients W 7.6% (10)	Patients WO 92.4% (122)	PD W 9.2% (19)	PD WO 90.8% (187)	Controls (463)	Patients total (524)	Patients W 48.7% (255)	Patients WO 51.3% (269)
A	23.2% (25)	25.7% (19)	11.1% (1)	27.7% (18)	25% (33)	20% (2)	25.4% (31)	15.8% (3)	26.2% (49)	41.7% (193)	17.9% (94)	10.2% (26)	25.3% (68)
B	19.4% (21)	29.7% (22)	88.9% (8)	21.5% (14)	25% (33)	80% (8)	20.5% (25)	84.2% (16)	20.9% (39)	13.2% (61)	57.3% (300)	79.6% (203)	36.1% (97)
C	32.4% (35)	33.8% (25)	(0)	38.5% (25)	35.6% (47)	(0)	38.5% (47)	(0)	38.5% (72)	31.5% (146)	16.6% (87)	1.2% (3)	31.2% (84)
D	25% (27)	10.8% (8)	(0)	12.3% (8)	14.4% (19)	(0)	15.6% (19)	(0)	14.4% (27)	13.6% (63)	6.9% (36)	8.2% (21)	5.6% (15)
Unknown	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	1.3% (7)	0.8% (2)	1.8% (5)

N number of chromosomes analyzed, *W* patients with *p.F508del* mutation, *WO* patients without *p.F508del* mutation, *PD* pooled data of Afro-Brazilian patients from Bahia and Minas Gerais States (p = 0.39)

[a]*XK* haplotypes: haplotype *A* formed by the alleles *XV2C-*1/KM19-*1*; haplotype *B* formed by the alleles *XV2C-*1/KM19-*2*; haplotype *C* formed by the alleles *XV2C-*2/KM19-*1*; haplotype *D* formed by the alleles *XV2C-*2/KM19-*2*; (*KM19-1** = absence of restriction site for PstI and *KM19-2** = presence of restriction site for PstI); (*XV2C-1** = absence of restriction site for TaqI and *XV2C-2** = presence of restriction site for TaqI)

[b]Data from Raskin et al. (1997a, b)

Table 2 Distribution of *XK* allele frequencies, haplotypes and genotypes in Afro-Brazilian chromosomes

XK loci alleles, haplotypes and Genotypes	Afro-Brazilians			
	Patients from BA	Patients from MG	Pooled data	Controls from MG
*XV2C-*1*	50% (66)	55.4% (41)	51.9% (107)	42.6% (46)
*XV2C-*2*	50% (66)	44.6% (33)	48.1% (99)	57.4% (62)
*KM19-*1*	60.6% (80)	59.4% (44)	60.2% (124)	55.6% (60)
*KM19-*2*	39.4% (52)	40.6% (30)	39.8% (82)	44.4% (48)
A[a]	25% (33)	25.7% (19)	25.3% (52)	23.1% (25)
B[b]	25% (33)	29.7% (22)	26.7% (55)	19.5% (21)
C[c]	35.6% (47)	33.8% (25)	34.9% (72)	32.4% (35)
D[d]	14.4% (19)	10.8% (8)	13.1% (27)	25% (27)
AA	3.0% (2)	8.2% (3)	4.8% (5)	7.4% (4)
AB	19.7% (13)	16.2% (6)	18.4% (19)	9.3% (5)
AC	19.7% (13)	16.2% (6)	18.4% (19)	13.0% (7)
AD	4.6% (3)	2.7% (1)	3.9% (4)	9.3% (5)
BB	4.6% (3)	10.8% (4)	6.8% (7)	3.7% (2)
BC	10.6% (7)	10.8% (4)	10.7% (11)	11.1% (6)
BD	10.6% (7)	10.8% (4)	10.7% (11)	11.1% (6)
CC	15.1% (10)	18.9% (7)	16.6% (17)	9.3% (5)
CD	10.6% (7)	2.7% (1)	7.8% (8)	18.5% (10)
DD	1.5% (1)	2.7% (1)	1.9% (2)	7.4% (4)

[a]Haplotype *A* formed by the alleles *XV2C-*1/KM19-*1*

[b]Haplotype *B* formed by the alleles *XV2C-*1/KM19-*2*

[c]Haplotype *C* formed by the alleles *XV2C-*2/KM19-*1*

[d]Haplotype *D* formed by the alleles *XV2C-*2/KM19-*2*

Analyzing the data of 108 non-CF chromosomes from Afro-Brazilians living in MG state (referred as Afro-Brazilian controls) with the pooled data of 206 CF chromosomes from Afro-Brazilians living in BA and MG state (referred as Afro-Brazilian patients) no statistically significant was found in the *KM19* and *XV2C* alleles ($p = 0.39$ and 0.099, respectively) (Table 2). The frequency of the *XK* haplotypes present in African/Afro-descendants patients is shown in Table 3.

Table 3 Distribution of *XK* haplotypes in African and Afro-descendants chromosomes

XK loci alleles and haplotypes	General population (controls)			Cystic fibrosis patients		
	Afro-Brazilians[a] (108)	African[b] (82)	Afro-American[c] (64)	Afro-Brazilians[d] (206)	Afro-American[c] (27)	Afro-American[e] (20)
*XV2C-*1*	42.6% (46)	41.5% (34)	53.1% (34)	51.9% (107)	55.6% (15)	55% (11)
*XV2C-*2*	57.4% (62)	58.5% (48)	46.9% (30)	48.1% (99)	44.4% (12)	45% (9)
*KM19-*1*	55.6% (60)	70.7% (58)	64.1% (41)	60.2% (124)	25.9% (7)	60% (12)
*KM19-*2*	44.4% (48)	29.3% (24)	35.9% (23)	39.8% (82)	74.1% (20)	40% (8)
A	23.2% (25)	15.9% (13)	25% (16)	25.3 (52)	7.4% (2)	NA
B	19.4% (21)	41.5% (34)	28.1% (18)	26.7% (55)	48.2% (13)	NA
C	32.4% (35)	17.1% (14)	39.1% (25)	34.9% (72)	18.5% (5)	NA
D	25% (27)	25.6% (21)	7.8% (5)	13.1% (27)	25.9% (7)	NA

N number of chromosomes analyzed, *NA* not available

[a]Afro-Brazilian controls from MG State

[b]African controls studied by Denter et al. (1992)

[c]Afro-American controls and patients studied by Cutting et al. (1989)

[d]Data pooled from Afro-Brazilian CF patients from BA and MG States

[e]Afro-American patients studied by Martin et al. (1988)

Discussion

As was expected, no *p.F508del* mutation was found in Afro-Brazilian controls studied here. Also, no significant difference of the frequency of this mutation was found in the two populations of Afro-Brazilian patients analyzed by this study and the same homogeneity between both populations was found regarding the *XK* haplotypes frequency. Even comparing the Afro-Brazilian controls with Afro-Brazilian patients (data pooled), no significant difference was found in the frequency of the *KM19* and *XV2C* alleles. But, when we compare the Afro-Brazilian with the Euro-Brazilian data the results show a great variation that could be explained by admixture of different ethnic groups found in Brazil.

Brazil has a population of more than 190 million people (Brazilian Institute of Geography and Statistics 2010) making it the fifth most populous country in the world, and shows regional variation of ethnic composition. Amerindians were autochthonous when Portugal conquered Brazil in 1500, and various other European, Middle Eastern, and Asian immigrant groups have settled in Brazil since the middle-nineteenth century. About 3.6 million Africans were brought as slaves to Brazil from 15th to nineteenth centuries (Klein 2002). Now, Brazilians of European origin makes up 48% of the total population, mixed populations 43%, Afro-Brazilians almost 8%, Brazilians of Asian origin 1% and Brazilian Amerindians almost 0.5% (Brazilian Institute of Geography and Statistics 2010).

The use of polymorphic DNA segments as genetic markers has greatly expanded the potential applications of linkage analysis and contributed to our knowledge of human genome variation and disease (Cooper and Clayton 1988). Usually in population association or case reference studies the gene in question or a nearby RFLP locus is genotyped to compare the allele frequencies seen in different populations. In CF, the distribution of *XK* haplotypes has been determined in several populations, but mainly in Euro-descendants CF patients (Cutting et al. 1989; Estivill et al. 1988, 1987a; Raskin et al. 1997a, b). Here we compared the data obtained from Afro-Brazilian controls with the data shown by Raskin et al. (Raskin et al. 1997a, b) on Euro-Brazilian controls where an extremely statistically significant difference in the *KM19* and *XV2C* alleles ($p < 0.001$ and 0.022, respectively) where found.When the *p.F508del* mutation Afro-Brazilian patients are compared with *p.F508del* mutation Euro-Brazilian patients (Raskin et al. 1997a, b) we did not observe a statistically significant difference in the *KM19* and *XV2C* alleles ($p = 0.57$ and 0.16, respectively) but for patients that have other mutations than *p.F508del* a statistically significant difference is observed for the *XV2C* allele ($p < 0.001$), although no statistically significant difference was found for *KM19* allele ($p = 0.074$) that could be influenced by the sample size. Comparing the data from Afro-Brazilian controls with African controls (Denter et al. 1992) (Table 3) we observe a statistically difference for the *KM19* allele ($p < 0.008$) but not for the *XV2C* allele ($p = 0.995$). There is no statistical difference for both *XK* alleles (KM19 $p = 0.138$ and *XV2C* $p = 0.103$) of Afro-Brazilians controls compared with Afro-American controls (Cutting et al. 1989) (Table 3). An extremely difference in the *KM19* allele frequency is found comparing Afro-Brazilian patients with Afro-American patients studied by Cutting et al. (Cutting et al. 1989) ($p < 0.001$) (Table 3), but there is no difference in the *XV2C* allele frequency between these two populations ($p = 0.758$). There is no statistically difference in both *KM19* ($p = 0.960$) and *XV2C* ($p = 0.824$) alleles comparing Afro-Brazilian patients with Afro-American patients studied by Martin et al. (Martin et al. 1988) (Table 3).

Specific haplotypes are non-randomly associated with specific CF alleles. In all the CF Euro-Brazilian populations tested so far, the haplotype B is strongly associated with CF bearing chromosomes, but is relatively rare on non-CF-bearing chromosomes (Cabello et al. 2005; Raskin et al. 2007, 1997a). The frequency of chromosomes linked to the B haplotype on non-CF-bearing chromosomes is 41.5% in Africans (Denter et al. 1992), 28.1% in Afro-Americans (Cutting et al. 1989) and 19.4% in Afro-Brazilian as presented here (Table 3). We have previously showed that CF B haplotype is present in 17% of non-CF chromosomes from Euro-Brazilians living in Paraná (PR) and in 18% of non-CF chromosomes from Euro-Brazilians living in MG (Raskin et al. 1997b), and therefore, they do not differ in the frequencies of the B haplotype. However in CF bearing chromosomes, the B haplotype was found in 48.2% of Afro-Americans (Cutting et al. 1989) and in 26.7% of Afro-Brazilians studied here, much lower than the results previously found in Euro-Brazilians CF patients living in PR where the CF B haplotype was present in 71% of CF chromosomes and in 89.5% CF *p.F508del* chromosomes (Raskin et al. 1997b). This haplotype is also present in 50% of CF chromosomes and in 89.3% CF *p.F508del* chromosomes from Euro-Brazilian CF patients living in MG (Raskin et al. 1997a, b) showing a strong association of *p.F508del* mutation with the B haplotype in CF Euro-descendant patients. This also supports the hypothesis that there was a single origin for *p.F508del* mutation, in the European ancestors after the divergence of continental groups, around 52,000 years ago during the Paleolithic Age (Kerem et al. 1989; Morral et al. 1994).

In this study we show that CF B haplotype is present in Afro-Brazilians CF patients from the state of MG in only 29.7% of the total CF chromosomes and in almost 89% of *p.F508del* chromosomes. In the state of Bahia, we found the haplotype B in 25% of Afro-Brazilian CF chromosomes

and in 80% of the *p.F508del* chromosomes from Afro-Brazilian patients (Table 1). The low frequency of *p.F508del* found in Afro-Brazilians CF patients as shown by this study (12.2% for MG and 7.6% for BA) is similar to what was found by Costa et al. (Costa et al. 2007) in BA state (8.7%). These data clearly differ from the frequency of this mutation in Euro-Brazilians (48.7%) (Table 1) (Raskin et al. 1997a, b). In non-*p.F508del* CF chromosomes, 20.9% were linked to the B haplotype in Afro-Brazilians and 36.1% in Euro-Brazilians. The data show that in Afro-Brazilians non-*p.F508del* mutations are occurring with the haplotypes C (38.5%) and A (26.2%) most frequently than the B haplotype (Table 1). Therefore, the association of the haplotype B and the *p.F508del* mutation in Afro-Brazilians could be considered as a marker of admixture with Euro-descendants and maybe it could be applied for other populations, like the Brazilian Amerindians, in which neither the *p.F508del* mutation nor the B haplotype were found (Raskin et al. 2007), findings in agreement with the low admixture rate between Brazilian Amerindians and Euro-descendants.

In Euro-Brazilians the frequency of *p.F508del* mutation is 48.7%; in Afro-Brazilians from MG State (where the admixture rate between Afro-Brazilians and Euro-descendants is 50%) (Salzano and Freire-Maia 1967), its frequency is 12.2% and in Afro-Brazilians from BA State (where more than 76% of the population is descendant from Africans) (Brazilian Institute of Geography and Statistics 2010) the frequency of *p.F508del* is 7.6%. Although the haplotypes distribution found demonstrates no clear-cut differences between non-CF Afro-Brazilians when compared to non-CF Euro-Brazilians from PR and MG, in Afro-Brazilians CF patients only 46.6% of the chromosomes had A/B, B/B, B/C and B/D *XK* genotypes (Table 2). The sum of the four B genotype frequencies is lower than in chromosomes of CF Euro-Brazilians patients (68%) (Raskin et al. 1997a, b), lower than the CF *XK* African-American data (71.4%) (Cutting et al. 1989) and even lower than chromosomes of Euro-Brazilians controls (58.4%) (Raskin et al. 1997a, b), suggesting that the B haplotype is present in the Afro-Brazilian CF population due to admixture with Euro-descendants.

The differences in the frequencies of the B haplotype and *p.F508del* CF chromosomes between Afro-Brazilians and Afro-Americans CF patients found in this and previous work (Raskin et al. 2003) are not surprising, as historical, social and genetics data show the differences between Afro-descendants from North and South America; indeed, mitochondrial DNA data (Salas et al. 2004) shows that the largest Afro component in North America appears to derive from Western Africa, while in South America, the largest contribution of Africans came from West-Central Africa. This, and the different admixture rates between African and non-African descendants in North and South America (Salzano and Freire-Maia 1967) may contribute to explain the differences found in the *p.F508del* mutation and the B haplotype CF chromosome frequencies between these two Afro-descendants populations.

We found haplotype differences between Afro-Brazilians and Euro-Brazilians CF patients: in Afro-Brazilians CF patients the A and C haplotypes occur in similar rates as the B haplotype whereas in Euro-Brazilians CF patients the B haplotype rate is more than three times higher than the A and C haplotypes (Table 1). We hypothesized that KM19-*2 allele (in part responsible for the B haplotype) is of relatively recent origin, being almost absent in the ancestors of Afro-Brazilians. Its frequency in Afro-Brazilians could be explained mainly by admixture of Euro-Brazilians with Afro-Brazilians, known to be around 50% in the state of MG (Salzano and Freire-Maia 1967). The KM19-*2 allele, and therefore the B haplotype, would have originated after divergence of continental groups, in the European population, probably by the time *p.F508del*, the first CF mutation, was introduced (Morral et al. 1994). The data about the frequency of the B haplotype in Brazilian Amerindians also agree with the hypothesis that the B haplotype would have originated after divergence of continental groups, in the European population (Raskin et al. 2007). Assuming that the presence of the *p.F508del* mutation in Afro-Brazilian is almost due to admixture with Euro-descendants, this mutation could be used as a marker of admixture between these two groups, and for other populations like Brazilian Amerindians (Raskin et al. 2007) or Afro-Americans (Cutting et al. 1989; Martin et al. 1988).

Further studies in larger samples are needed to compare other extragenic and intragenic *CFTR* polymorphisms as well as mutations in the *CFTR* gene in other Afro-Brazilians and Africans, bringing new insights concerning *CFTR* origin as well as help clarifying the history of CF in Brazil, especially because CF is one of the most common genetic disease in some states of Brazil, but it is still underdiagnosed (Raskin et al. 2008). In the last 15 years, with the development of medical centers specialized in the diagnosis and treatment of CF patients in Brazil, the number of diagnosed CF cases has increased. Although more than 2600 cases are registered in 2012 in the Brazilian CF Registers, only around 29.8% are Afro-Brazilians (6.4% considered as black and 23.4% as mixed-race) (Brazilian Study Group of Cystic Fibrosis 2012), considering that the mixed population in Brazil is about 43% (Brazilian Institute of Geography and Statistics 2010) the number of CF affected people must be higher, but is been underdiagnosed.

The immigration history and heterogeneity of the Brazilian population makes analysis of the geographic distribution of *CFTR* mutations and tightly linked RFLPs of particular interest. The careful analysis of *CFTR* mutations and polymorphism of the different ethnic groups is

an important step to understand its evolution and features. Recent advances in genetic screening using high-throughput approaches (like Next Generation Sequencing, NGS) and the crescent reductions in DNA sequencing costs allow a deeper analysis of the genetic causes of Cystic Fibrosis in African and Afro-descendant populations where the disease is supposedly rare.

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Compliance with ethical standards

Conflict of interest The authors report no conflict of interest.

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