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MC4-R variant confirms its association with obesity during progression from childhood to adolescence

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The rs12970134 variant near the melanocortin receptor 4 (MC4-R) has gained relevance suggesting an age dependent phenotypic effect in the induction of obesity in young age. A previous study evaluating 740 Caucasian children has shown this association in prepubertal children older than 8 years. The aim of this study was to assess whether the obesogenic effect of M4CR gene contributed to obesity also in adolescence. After 8 years participants of the original study were contacted and invited to perform an anthropometric evaluation. Out of 35 carriers of the AA risk allele of MC4-R, 12 subjects accepted to participate. Adolescent subjects with the AA risk allele of MC4-R were matched with 24 and 48 subjects, respectively for AG and GG variants. Differences between the three MC4-R genotypes for anthropometric data, for percentage of overweight and obesity and for changes in BMI-SDS over visit have been assessed. At Visit 1 (baseline examination study), the AA risk genotype was confirmed to be associated with higher BMI-SDS (1.3 ± 0.4 vs 0.4 ± 0.1) and waist circumference (66.5 ± 5.8 vs 60.9 ± 7.1) when compared to the GG genotype ($p < 0.016$ both). At Visit 2 the AA genotype not only was associated with a higher BMI-SDS (1.07 ± 0.5 vs 0.02 ± 0.8) and WC (95.6 ± 13.3 vs 64.9 ± 13.5) when compared to GG genotype, but also when compared to AG genotype (vs 0.5 ± 0.1 and 62.9 ± 10.0, $p < 0.016$). Whereas AA genotype demonstrated no change of BMI-SDS between visit 1 and visit 2 (p00.32), AG and GG genotype showed a significant reduction ($p = 0.01$ and 0.001 respectively). Furthermore, a higher percentage of patients were affected by overweight/obesity in the AA genotype compared to AG and GG genotypes (50% vs 20.8% vs 16.5% $p = 0.03$). This study demonstrates that the rs12970134 variant not only exerts an obesogenic influence in the prepubertal age but remains a major risk factor also during adolescence.

Keywords MC4-R, Obesity, Overweight, Adolescents, MC4-R risk allele

Puberty is typically associated with major changes in lifestyle and represents a significant period of metabolic transition. During this phase, a physiological insulin resistance occurs as part of normal pubertal development, which is distinct from the pathological insulin resistance associated with obesity. The latter is exacerbated by excess adiposity and metabolic dysfunction, increasing the risk of long-term cardiometabolic complications[1]. Recent epidemiological studies indicate that up to 20% of adolescents in Western societies are overweight or obese. This risk seems to be related to the increase of obesogenic environmental influences, including excess intake of calorie-dense foods combined with a substantial lack of physical activity. In this context the individual genetic background plays a key role, where a favoring genetic architecture might lay the ground for the development of obesity[2]. Genome-wide association studies have identified several genetic variants, primarily single-nucleotide polymorphisms (SNPs), that are associated with obesity. Among these, the rs12970134 variant near the melanocortin receptor 4 (MC4-R) has gained increasing attention, as multiple studies suggest its age-dependent phenotypic effect on obesity risk[3]. Specifically, MC4-R seems to play a major role in the induction of obesity in young age when compared to older people[4]. In this context, the pediatric population represents a critical period for studying the genetic influences on obesity, as research has shown that the heritability of obesity traits is stronger during childhood and adolescence. Additionally, this life stage is characterized by significant physiological and behavioral changes, which may modulate the effects of specific genetic variants over time[5]. In fact, in a former study we demonstrated an age dependent effect of the MC4-R genotype on obesity expressing

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itself more importantly in prepubertal children although older than 8 years[5]. Thus, whether this peculiar age dependent effect persist later in early life as not yet been documented. Therefore, the aim of this follow-up study was to determine whether the obesogenic effect of MC4-R persisted also during puberty.

Methods

Study design

In the original study a homogeneous prepubertal population of 740 Caucasian children attending different Primary Schools of Chieti had been evaluated and the association between the gene haplotype AA of the MC4-R genes variants with adiposity had been detected[5]. To further ensure population homogeneity, we also analyzed participants' surnames, as previous studies have validated this method as a proxy for regional ancestry and familial background. After 8 years participants of the original study were contacted by telephone call and invited to perform a further anthropometric evaluation at the Outpatient Department of Pediatrics of the University of Chieti. All 35 carriers of the AA risk allele of MC4-R were invited out of them 23 subjects declined participation while 12 subjects accepted. Subjects with the AA risk allele of MC4-R were subsequently matched with subjects of the original database on the grounds of original primary school, age and gender. 70 subjects were identified for the AG risk allele and 120 for the GG risk allele. All subjects were subsequently contacted by telephone call and recruitment was stopped when 24 and 48 subjects respectively for AG and GG variants accepted to participate. The Research Ethics Committee of the University of Chieti approved this study. Written informed consent was signed by the parents and oral assent from the children involved.

Anthropometric measurements

Anthropometric measurements were obtained in each patient, along with obesity indices. Height was measured to the nearest 0.1 cm with Harpenden stadiometer. Body Weight was measured to the nearest 0.1 kg with a calibrated scale. A flexible tape was used to measure waist circumference to the nearest 1 mm; WC was measured at the mid-point between the lower ribs and the pelvic bone.

Body Mass Index (BMI), used as index of adiposity was calculated as the weight in kilograms divided by the square of height in meters (kg/m^2). BMI standard deviation score (BMI-SDS) was calculated based on the age and sex reference values for the Italian Population[6]. Overweight and obesity were defined based on BMI-SDS thresholds from the Italian growth charts by Cacciari et al.[6]. Specifically, overweight was classified as BMI-SDS > 1.0364, and obesity as BMI-SDS > 1.6448. Overweight and obesity categories were defined as BMI-SDS above 1.0364 and 1.6448, respectively.

Blood pressure was measured using a validated protocol. Systolic and diastolic blood pressures (SBP and DP) were measured three times, at 5 min intervals at the nondominant arm after a 10 min rest using a calibrated sphygmomanometer. The mean of the three measurements was taken as the individual SBP and DBP.

Statistical analysis

Data have been expressed as mean ± standard deviation or standard error. Differences between the 3 MC4-R genotypes (AA, AG, GG) have been evaluated with Kruskal Wallis testing and values of $p \leq 0.05$ have been considered significant while Mann Whitney test has been applied for post-hoc comparison between groups and p values ≤ 0.16 have been accepted as significant. Differences in terms of overweight and obesity between the three groups have been assessed through chi-square analysis. Furthermore, differences in terms of changes in BMI-SDS over time within the three groups was analyzed by Wilcoxon test and values for $p \leq 0.05$ were considered significant. Statistical analysis has been performed with SPSS, version 22.0 for Windows.

Results

The follow-up period was 8 years, with subjects evaluated initially at a prepubertal stage and subsequently during puberty. 12 carriers of the AA genotype were enrolled and compared with a group of 72 subjects homogeneous for age and sex (24 carriers of the AG genotype and 48 carriers of the GG genotype). The mean age at visit 1 was 8.8 ± 0.9 years (genotypes AA), 8.7 ± 1.2 (genotypes AG), and 8.6 ± 1.3 (genotypes GG). At visit 2 the mean age was 16.8 ± 0.9 years (genotypes AA), 16.7 ± 1.3 years (genotypes AG), and 16.7 ± 1.3 years (genotypes GG). All subjects were classified as adolescents, rather than strictly post-pubertal, since menarche in females and voice deepening in males occur at various stages of pubertal development. Therefore, obesity estimation in this study is based on adolescent status rather than confirmed post-pubertal maturity[7].

Clinical and anthropometric characteristics of visit 1 and visit 2 are listed in Table 1. Visit 1 and visit 2 represent, respectively, baseline examination and the visit after 8 years. No significant differences were found between the three MC4R genotype groups in terms of age, birth weight, or blood pressure values for both time points.

At visit 1 we confirmed a progressive increase of BMI-SDS and waist circumference across the rs12970134 genotypes ($p = 0.008$ and 0.016 respectively). In fact, also in this subset of data, subjects with the AA genotype had a significantly higher BMI-SDS compared with GG genotype (1.3 ± 0.4 vs 0.4 ± 0.1 $p < 0.008$) and an increased WC (66.5 ± 5.8 vs 60.9 ± 7.1 $p < 0.016$).

At visit 2, subjects with AA genotype presented a significant higher BMI-SDS not only when compared to GG genotype but also to AG (1.07 ± 0.5 vs 0.02 ± 0.8 and 0.2 ± 0.9, $p < 0.009$ both). This was associated with a significantly larger WC for AA genotype when compared to both AG and GG (95.6 ± 13.4 vs 81.7 ± 10.1 and 64.9 ± 13.5 $p < 0.01$ for both). Whereas the AA genotype showed no change in BMI-SDS between visit 1 and visit 2 ($p = 0.32$), AG and GG genotypes showed a reduction in BMI-SDS ($p = 0.01$ and 0.001, respectively) (Fig. 1).

As in our previous study, overweight and obese children were considered as a single category to assess the association between MC4R and adiposity.

Variable	Genotypes			
	AA	AG	GG	*p* for trend
Number of subjects	12	24	48	
Gender (male/female)	5/7	12/12	25/23	0.51
Age visit 1 (years)	8.8 ± 0.9	8.7 ± 1.2	8.6 ± 1.3	0.11
Age visit 2 (years)	16.8 ± 0.9	16.7 ± 1.3	16.7 ± 1.3	0.09
BW (gr)	3410 ± 466	3291 ± 394	3189 ± 489	0.11
WC visit 1 (cm)	66.5 ± 5.8	62.9 ± 10.0	60.9 ± 7.1	0.016[a]
WC visit 2 (cm)	95.6 ± 13.4	81.7 ± 10.1	64.9 ± 13.5	0.01[ab]
BMI visit 1 (kg/m^2)	20.9 ± 3.5	19.2 ± 3.7	18.9 ± 3.2	0.14
BMI visit 2 (kg/m^2)	24.7 ± 4.1	21.4 ± 3.5	21.4 ± 3.4	0.02[ab]
BMI SDS visit 1	1.3 ± 0.4	0.5 ± 0.1	0.4 ± 0.1	0.008[a]
BMI SDS visit 2	1.07 ± 0.5	0.2 ± 0.9	0.02 ± 0.8	0.009[a]
Overweight/obese visit 1 (N, %)	9 (75%)	6 (25%)	14 (29.1%)	0.02
Overweight/obese visit 2 (N, %)	6 (50%)	5 (20.8%)	8 (16.6%)	0.03
SBP mmHg visit 1	104 ± 9	103 ± 9	103 ± 10	0.42
SBP mmHg visit 2	108 ± 8	107 ± 7	105 ± 8	0.33
DBP mmHg visit 1	62 ± 6	61 ± 6	62 ± 6	0.42
DBP mmHg visit 2	72 ± 4	72 ± 5	71 ± 5	0.62

Table 1. Clinical and anthropometric characteristics of included patients. [a]AA versus GG. [b]AA versus AG.

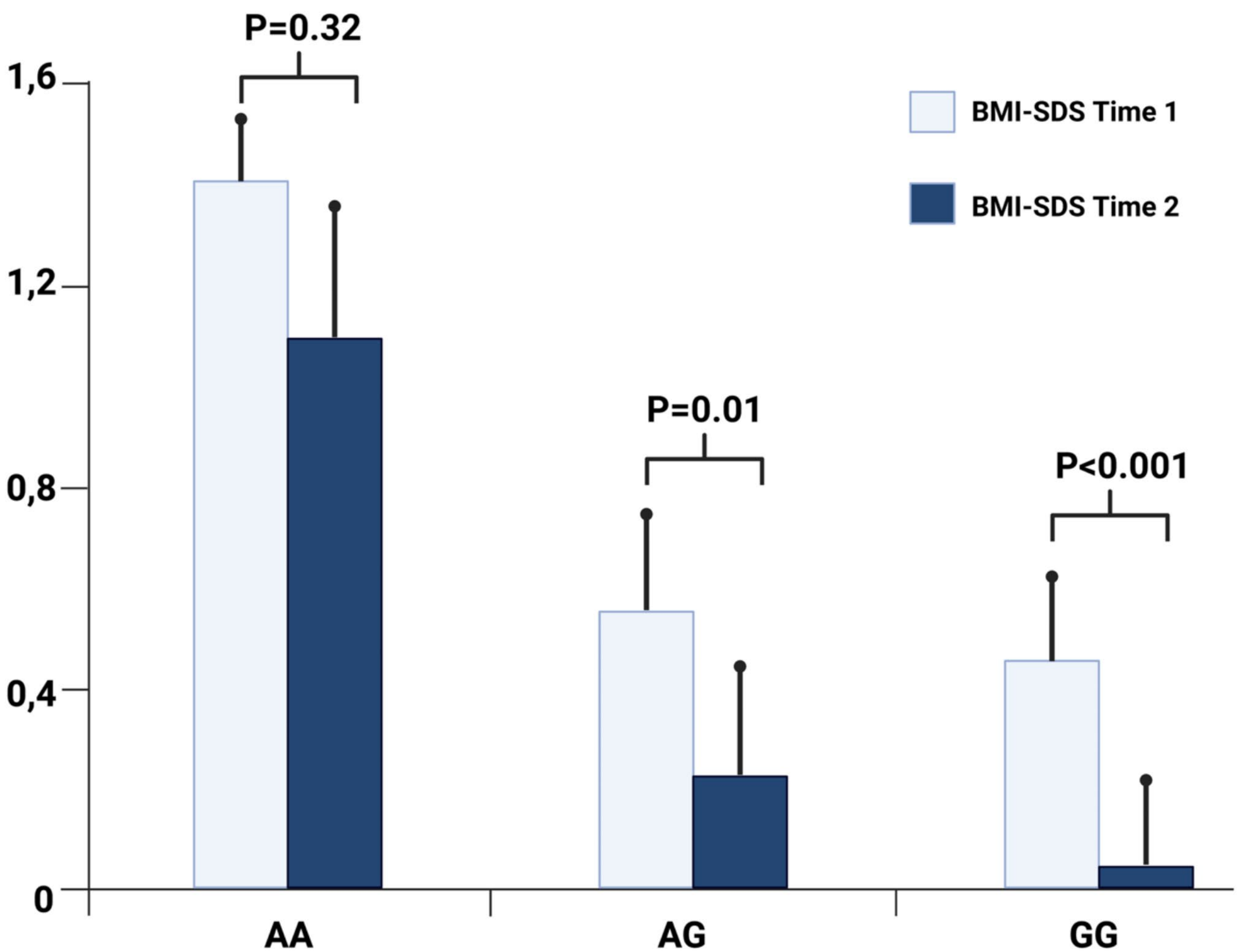

Fig. 1. Results. AA genotype showed no change in BMI-SDS between visit 1 and visit 2 ($p = 0.32$), whereas genotypes AG and GG demonstrate a significant decrease in BMI-SDS ($p = 0.01$ and 0.001 respectively).

Although the prevalence of obesity and overweight decreased in all genotypes between visit 1 and at visit 2 the AA genotype still showed a higher percentage of obese/overweight (50%) individuals compared to AG (20.8%) and GG genotypes (16.6%), as reported in Table 1.

Discussion

This small follow up study demonstrates that the rs12970134 variant near the melanocortin receptor 4 (MC4-R) not only explicates an obesogenic effect in the prepubertal age but remains a major risk factor also during post pubertal age. Pubertal development is known to influence BMI-SDS due to changes in body composition, hormonal regulation, and metabolic adaptations occurring during adolescence. Previous studies have shown that pubertal insulin resistance, increased adiposity redistribution, and growth spurts may differentially impact BMI-SDS trajectories across genetic backgrounds[8]. While our study matched post-pubertal subjects, individual variability in pubertal timing could still represent a confounding factor in BMI-SDS comparisons between genotypes. MC4-R, an alfa-MSH receptor, whose function is to induce feeding related satiety and increase of energy expenditure is highly expressed in the central nervous system[9]. In fact, loss of function mutations of MC4-R was among the first ones to be associated with severe obesity[10]. It has been suggested that these mutations represent the most common cause of human monogenic obesity, being involved in 4% of obese individuals[8]. To date, several large population studies have been carried out to test this relationship, both in the adult and in the pediatric age[11]. In fact, Farooqi and colleagues, showed that MC4-R SNP was associated in adults with hyperphagia, visceral obesity as well as hyperinsulinemia, high blood triglyceride and LDL cholesterol levels[12]. Paediatric studies detected higher adiposity indexes in subjects carrying the risk allele compared to non-carriers[10]. Specifically, studies asses that individuals possessing MC4R gene variants consume increased amounts of food enriched with total and saturated fatty acids. Therefore, children possessing MC4R gene variants have an increased preference for calorie-dense foods enriched with fat, in addition to a decreased propensity for energy expenditure, both of which promote weight gain[13].

However, several studies suggested that the phenotypic effect of MC4-R might be age dependent[5]. These studies demonstrated that the progress of this relationship is biphasic with a greater influence during the first 20 years of life and a progressive decline of this association during adulthood and old age[14]. In a longitudinal study conducted by Bjørnland and collaborators it has been shown that within the geriatric age this association became increasingly weak[15]. A similar study by Hardy et al. showed that this ratio becomes progressively weaker 3/11/2025 8:00:00 AMalso in the transition between pediatric age and adulthood[14]. Furthermore, this might be true also within the pediatric age as in in our original study of only prepubertal children we documented an age dependent effect of MC4-R[5]. In fact, the association with adiposity indexes was stronger in subjects older than 8 years. This longitudinal study, for the first time, was conducted to assess the effect of MC4-R on adiposity indexes and obesity during puberty. Adolescence carrying the AA genotype presented higher values of adiposity indexes especially waist circumference suggesting an age dependent effect within the pediatric age group. This persistence of obesity in AA genotype might be explained by an influence of genetically driven environmental exposures such as the individual's behavior towards food or attitude towards physical activity[5]. Our findings suggest that genetic predisposition interacts with environmental factors, particularly feeding behaviors, rather than acting independently. This is supported by the fact that not all children carrying the AA genotype developed obesity at an early age, indicating that additional influences, such as dietary intake and lifestyle habits, modulate the obesogenic effect of the variant[12]. This age-dependent effect might start in elementary school but might be more relevant for adolescents who experience their first family independent choices of foods and sports. In this respect, the pediatric age group represents a unique model for observing gene-environmental interactions. Overall, these data are of major clinical relevance, as they show that MC4-R SNP predisposes to the accumulation of visceral obesity which, is undoubtedly associated with an increased metabolic and cardiovascular risk in the following years[16]. In contrast subjects with AG and GG genotype had a significant reduction in BMI-SDS over time and a significant lower waist circumference compared to AA genotype implying a better cardiovascular metabolic profile for the future.

In contrast to Huvenne[8] and collaborators who demonstrated an association between the MC4-R SNP and increased values of systolic and diastolic blood pressure, this association was not found in our study population. The discrepancy observed in our results may be influenced by multiple factors. First, differences in sample size and population characteristics between our study and previous research could contribute to the variation in findings. Second, methodological differences, including variations in BMI-SDS calculation methods or follow-up duration, might play a role. Lastly, environmental and lifestyle factors, such as dietary habits and physical activity levels, could have affected weight trajectories differently across cohorts. Future research should aim to standardize methodology and include larger, more diverse populations to resolve this inconsistency. This study has some limitations. The main limitations of this study are related to the low size sample and to the absence of metabolic data. Second, BMI-SDS analysis was not stratified by sex, despite the influence of gonadal hormones and pubertal timing on body composition. Future studies should consider sex-specific BMI-SDS trajectories to better assess these effects. Third, data on caloric intake and lifestyle habits, which significantly change during adolescence, were not collected. Given the role of diet, physical activity, and sedentary behavior in weight trajectory, their interaction with genetic predisposition should be investigated[10,17]. Fourth, hyperphagia was not assessed, limiting our understanding of its role in weight gain among MC4R carriers. Lastly, overweight and obesity were analyzed as a single category to increase statistical power, which may have masked differences in weight trajectory, particularly among adolescents transitioning between weight categories. Future studies should use a longitudinal design to track individual weight trends more accurately. A strength of the study is the case to control ratio of 1:2:3 allowing a double. An additional strength of this study is the homogeneity of the study population in terms of ethnicity and socio-cultural background. This was confirmed by our methodological approach, which included surname analysis to assess regional ancestry consistency. All surnames were typical of

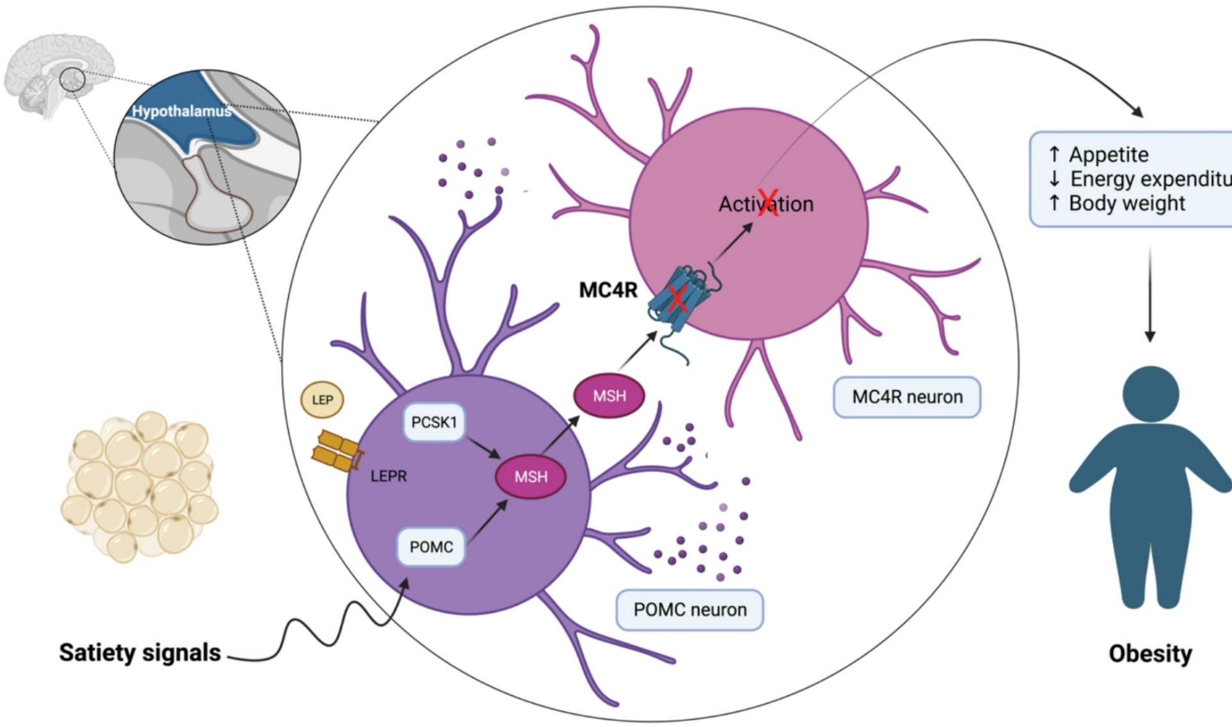

Fig. 2. Hypothalamic MC4R pathway with MC4R as a crucial mediator of appetite, energy expenditure, and body weight.

Chieti's area or of other Abruzzo's cities. Several studies have validated the surname use as a method of evaluation of population homogeneity. Moreover, all subjects continued to live within their original families and continued to attend local schools.

Conclusion

In conclusion, this study highlights the importance of the genetic background on the acquisition of body weight demonstrating the persistence of an obesogenic effect of MC4-R through puberty (Fig. 2). This aspect is important in clinical practice since it reduces the weight of environmental factors on obesity and offers preventing strategies in those carrying the risk genotype.

Data availability

All data generated or analysed during this study are included in this published article.

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Author contributions

C.G. and A.M. wrote the manuscript, realized the table, coordinated, reviewed, and approved the final version of the manuscript. A.D.L. wrote the manuscript and realized the figure. F.L., G.D.P., and N.P. wrote the manuscript. F.C. coordinated, reviewed, and approved the final version of the manuscript. All author have read and agreed to the published version of the manuscript. All authors take full responsibility for the integrity and accuracy of all aspects of the work.

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Declarations

Competing interests

The authors declare no competing interests.

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Additional information

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