Melanocortin-4 receptor and proopiomelanocortin: Candidate genes for obesity in domestic shorthair cats

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SHORT COMMUNICATION

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Melanocortin-4 receptor and *proopiomelanocortin*: Candidate genes for obesity in domestic shorthair cats

Abstract

Obesity is an escalating global health problem affecting both humans and companion animals. In cats it is associated with increased mortality and multiple diseases, including diabetes mellitus. Two genes coding for proteins known to play a critical role in energy homeostasis across species are the proopiomelanocortin (*POMC*) gene and the melanocortin-4 receptor (*MC4R*) gene. A missense variant in the coding sequence of the feline *MC4R* (*MC4R*:c.92C>T) has been reported to be associated with diabetes and overweight in domestic shorthair cats, and while variants in the *POMC* gene are known to cause obesity in humans and dogs, variants in *POMC* and their association with feline obesity and diabetes mellitus have not been investigated to date. The current study aimed to assess the association between the previously described *MC4R* variant and body condition score (BCS), as well as body fat content (%BF) in 89 non-diabetic domestic shorthair cats. Furthermore, we investigated the feline *POMC* gene as a potential candidate gene for obesity. Our results indicate that the *MC4R*:c.92C>T polymorphism is not associated with BCS or %BF in non-diabetic domestic shorthair cats. The mutation analysis of all *POMC* exons identified two missense variants, with a variant in exon 1 (c.28G>C; p.G10R) predicted to be damaging. The variant was subsequently assessed in all 89 cats, and cats heterozygous for the variant had a significantly increased body condition score ($p=0.03$) compared with cats homozygous for the wild-type allele. Results from our study provide additional evidence that the previously described variant in *MC4R* is not associated with obesity in domestic shorthair cats. More importantly, we have identified a novel variant in the *POMC* gene, which might play a role in increased body condition score and body fat content in domestic shorthair cats.

A obesity is currently one of the greatest health and welfare challenges facing cats globally, with as many as 63% of cats reported to be overweight (Wall et al., 2019; Wallis & Raffan, 2020). Many studies have shown that excessive adiposity negatively impacts feline health owing to its attendant comorbidities including an increased risk of developing diabetes mellitus (Clark & Hoenig, 2021; German, 2006). Overweight and obesity are known to be multifactorial disorders caused by an imbalance between food intake, nutrient composition of the food and exercise (Maes et al., 1997). While long believed to be mainly caused by environmental factors and individual behaviours, evidence shows that obesity is the result of the interaction of environmental and genetic factors (Graff et al., 2017; Justice et al., 2017; Loos & Yeo, 2022; van Vliet-Ostaptchouk et al., 2009). Among the first genes identified to be associated with obesity were *melanocortin 4 receptor* (*MC4R*; Huszar et al., 1997; Vaisse et al., 1998; Yeo et al., 1998) and *pro-opiomelanocortin* (*POMC*; Krude et al., 1998; Yaswen et al., 1999). Both genes are part of the hypothalamic pathway (Loos & Yeo, 2022), a signalling network involved in the regulation of food intake and energy homeostasis (Hill, 2010; Meister, 2000; Millington, 2007; Timper & Brüning, 2017). Activation of MC4R reduces food intake and increases energy expenditure (Ghamari-Langroudi et al., 2015; Razquin et al., 2011; Wallis & Raffan, 2020), and variants in this gene have been shown to cause common forms of monogenic obesity in humans (Chami et al., 2020; Doulla et al., 2014; Valli-Jaakola et al., 2006). In contrast, POMC undergoes proteolytic cleavage to produce several neuroactive peptides (Wallis & Raffan, 2020), with the neuropeptide α-MSH being a predominant activator of the MC4R (Millington, 2007). Variants in the *POMC* gene have been reported to cause severe, early-onset obesity in humans as well as adiposity in Labrador Retrievers (Huszar et al., 1997; Raffan et al., 2016; Yang & Xu, 2020). In cats, a variant in the feline *MC4R* gene has been

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associated with diabetes mellitus in overweight domestic shorthair (DSH) cats (Forcada et al., 2014). Despite its integral role, the association between variants in *POMC* and feline obesity has not been investigated to date. The aim of this study was to assess the association between the previously described variant in *MC4R* and body condition score (BCS), as well as body fat content (%BF) in non-diabetic DSH cats. Furthermore, we investigated the *POMC* gene as a candidate gene for feline obesity.

Genomic DNA was isolated using a standard phenol–chloroform extraction method from EDTA-stabilised blood of 89 intact DSH cats belonging to a previously described breeding colony (Häring et al., 2011, 2013). Cats were maintained according to local animal welfare legislation and experiments were approved by the Ethics Committee for Animal Experiments (Veterinaeramt des Kantons Zurich, licence number 180/2009). As BCS at 8 months has previously been identified as a useful marker (Häring et al., 2013), the BCS of each cat was assessed at the age of 8 months according to the nine-point BCS system (Laflamme, 1997) and %BF was determined in 70 cats using dual energy X-ray absorptiometry (DEXA) as described before (Häring et al., 2013). Genotypes for the *MC4R*:c.92C>T variant were determined by PCR-RFLP as previously described (Forcada et al., 2014). Primers for each of the exons including exon flanking regions were designed for the feline *POMC* gene using Primer 3 (Table S1). For *POMC* variant detection, seven animals were randomly selected, and PCR products amplified in 20 µl reactions using AmpliTag Gold Taq polymerase (ThermoFisher). The yield and quality of PCR products were checked on an agarose gel. Direct sequencing of PCR products was performed after shrimp alkaline phosphatase and exonuclease treatment, using both PCR primers as sequencing primers. *POMC* sequences were compared with the feline reference sequences (FelCat9) using SEQUENCHER v5.4.6 (GeneCodes) and the functional effects were predicted using PolyPhen2. All animals including genotypes are listed in Table S2. Statistical analysis was performed using RCOMMANDER software package (version 4.2.1) and IGOR PRO (version 8.0.4.2). A simple linear regression analysis was performed to investigate the relationship between BCS and %BF. Genotypes were analysed for association with BCS and %BF using one-way ANOVA. *Post hoc* pairwise Tukey tests were used to identify significant differences between pairs. The threshold for significance was $p < 0.05$.

The mean BCS in the studied population was 5.6 (SD ±0.5) and the mean %BF was 13.2 (SD ±5.1). The BCS and %BF showed a positive linear correlation ($r = 0.782$), with an increase of body fat content by 7.34% (SEM = 1.39%) per unit increase in BCS (Figure 1). In contrast to the nine-point BCS system, which is a subjective assessment of the amount of body fat, DEXA is a machine and software-based technique measuring body composition. While DEXA measurements are influenced by several factors, the results have been shown to correlate well with those of the nine-point BCS system (Bjornvad et al., 2011, 2017), making both suitable methods for evaluating a cat's body condition. Our results demonstrate that the BCS and %BF measured by DEXA are highly correlated. This finding aligns with results from previous studies, where increases in %BF between 5 and 9.5% per unit in BCS have been observed (Bjornvad et al., 2011, 2017; Häring et al., 2013). Considering an average 5 kg cat, a 7.34% increase in %BF corresponds to a 367 g increase in body weight. This highlights the importance of closely monitoring body weight and/or BCS in

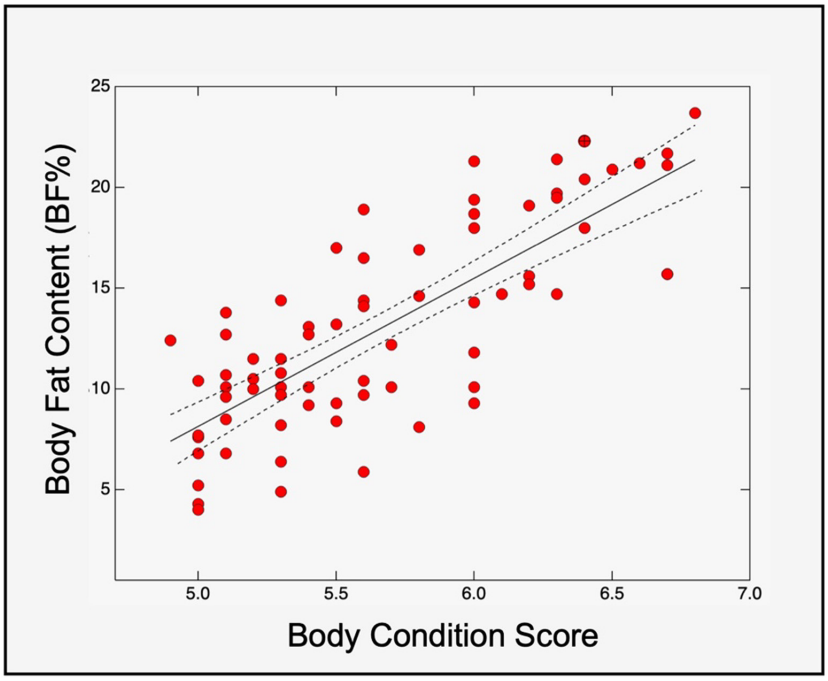

FIGURE 1 Correlation between body condition score and body fat content (BF%). Correlation between body condition score (BCS) assessed using the nine-point scheme by LaFlamme and body fat content (%BF) measured with dual X-ray absorptiometry (DEXA). The solid line represents the best fit and the dotted lines the 95% confidence interval.

preventing feline obesity as such small increments can be easily missed.

When the *MC4R*:c.92C>T variant was assessed in all 89 animals, 9% were homozygous for the reference allele, 36% were heterozygous and 56% were homozygous for the alternative allele, resulting in a minor allele frequency of 0.27. No significant differences between BCS and *MC4R*:c.92 genotypes could be observed (Figure 2a). Similarly, *MC4R*:c.92 genotypes were not significantly associated with %BF (Figure 2c). Similar to the original study (Forcada et al., 2014), we did not find an association between the *MC4R*:c.92 variant and BCS or %BF. Hence our findings support previous conclusions that while this variant may play a role in the development of diabetes mellitus, it is unlikely that it is responsible for obesity in cats. Given that several associations between *MC4R* variants and obesity have been described in humans (Alizadeh et al., 2022; Doulla et al., 2014; Fernandez et al., 2023; Huszar et al., 1997), and that the *MC4R* gene is located in one of the strongest susceptibility loci for obesity (Adamska-Patruno et al., 2021), this finding may seem unexpected. Variants in *MC4R* are the most common cause of monogenic obesity in humans and functional studies have confirmed the importance of MC4R signalling in maintaining energy homeostasis (Baldini & Phelan, 2019; Collet et al., 2017; Farooqi et al., 2003; Ghamari-Langroudi et al., 2015). However, findings from studies that investigated the association between *MC4R* variants and obesity are not always conclusive. While some studies identified variants near or within *MC4R* to be associated with appetite and energy intake (Qi et al., 2008; Wang et al., 2017), others did not find any associations with dietary intake (Hasselbalch et al., 2010) or body weight (Muller et al., 2014).

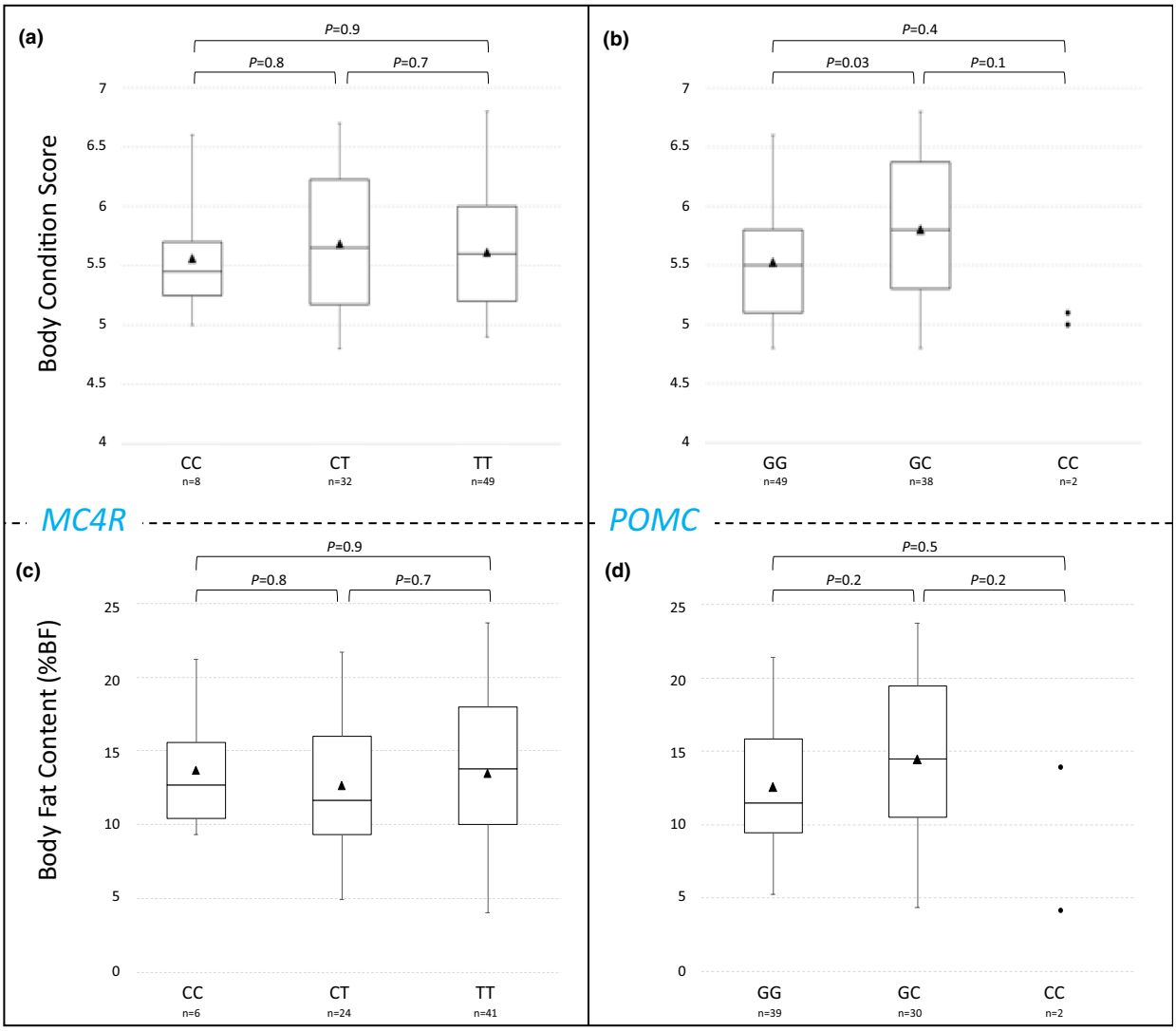

FIGURE 2 Association between phenotypes (body condition score and body fat content %) and genotypes at *MC4R* and *POMC*. Boxplot showing the genetic effect of (a) MC4R:c.92C>T on BCS, (b) POMC:c.28G>C on BCS, (c) MC4R:c.92C>T on %BF and (d) POMC:c.28G>C on %BF. Boxes indicate the 25th and 75th percentiles of each group. The horizontal line denotes the median; the triangle within the box plot indicates the mean; whiskers indicate values within the 1.5 interquartile range of the 25th and 75th percentiles for each group.

TABLE 1 Variants identified in the feline *POMC* gene.

Gene	Polymorphism (genomic DNA)[a]	Polymorphism (cDNA)[b]	Position within gene[c]	Protein	PolyPhen2
POMC	g.chrA3:123 050 469G>C	c.28G>C	Exon 1	p.G10R	Damaging
	g.chrA3:123 053 117G>A	c.524G>A/c.524A>G	Exon 2	p.G175E	Benign
	g.chrA3:123 053 120G>C	c.528G>C	Exon 2	Silent	
	g.chrA3:123 053 168C>T	c.576C>T	Exon 2	Silent	

[a]Genomic positions are according to FelCat9.
[b]Position according to XM_023252035.2.
[c]Position according to XP_023107803.2.

Functional assessment of *MC4R* variants indicated variant specific differences in MC4R signalling pathway activations (Paisdzior et al., 2020). As neither this nor the study by Forcada et al. (2014) identified an association between the *MC4R*:c.92C>T variant with BCS or %BF, the *MC4R*:c.92C>T variant may affect glucose homeostasis rather than body weight. This would align with findings from the original study, where an association between diabetes mellitus in overweight cats and *MC4R*:c.92C>T was observed (Forcada et al., 2014).

Comparative sequencing of the two coding *POMC* exons (accession numbers XM_023252035.2 and XP_023107803.2) including flanking regions identified a total of four variants, two synonymous and two missense variants (c.28G>C; c.524A>G) (Table 1). FelCat9 and F.catus_Fca126_mat1.0 alternate the reference alleles for the missense variant at *POMC*:c.524, with F.catus_Fca126_mat1.0 showing the FelCat9 alternative allele as the reference allele (Table 1). The resulting protein exchange was predicted to be benign. The *POMC*:c.28G>C (p.G10R) was consistent between the two reference assemblies. The variant is located within the highly conserved signal peptide sequence of *POMC* (Figure S1) and was predicted to be damaging (PolyPhen score 1.0). This variant was subsequently genotyped by direct sequencing of PCR products in all 89 cats, resulting in 55.1% homozygous ($n=49$) for the reference allele, 42.7% heterozygous ($n=38$) and 2.2% homozygous ($n=2$) for the alternative allele, resulting in a minor allele frequency of 0.24. When the association between *POMC*:c.28 genotypes and BCS was assessed, a significant difference between animals homozygous for the reference allele and heterozygous was identified ($p=0.03$; Figure 2b). While a similar trend was observed for %BF, the association between *POMC* genotypes and %BF was not significant (Figure 2d). Cats homozygous for the alternative allele in *POMC* were excluded from the analysis owing to the low genotype frequency ($n=2$; 2%) and the resulting lack of statistical power.

We identified a variant in *POMC* which might play a role in an increased body weight and body fat content in domestic shorthair cats. Several studies have investigated the role of POMC and its involvement in the control of feeding behaviours and energy homeostasis (Challis et al., 2004; Cowley et al., 2001; Saper et al., 2002). Proopiomelanocortin deficient humans and mice are hyperphagic, and display increased fat mass, while exhibiting a reduced energy expenditure (Krude et al., 1998; Tung et al., 2006; Yaswen et al., 1999; Zemel & Shi, 2000). Similarly, associations between variants in *POMC* and obesity in dogs have been reported (Sheet et al., 2020; Wallis & Raffan, 2020), with a frameshift mutation in the *POMC* gene shown to be associated with an increased food intake in Labrador Retrievers (Mankowska et al., 2017; Raffan et al., 2016). While the association between the *POMC*:c.28G>C variant and food intake in cats is yet to be established, cats of the investigated breeding colony and classified as overweight at the age of 8 months have previously been shown to exhibit higher food intake (Ghielmetti et al., 2018).

The leptin–melanocortin signalling pathway is highly conserved across species (Garcia & Shaw, 2017), suggesting that variants in *POMC* coding sequence are likely to have similar effects in various species. The variant identified in this study is located within the signal peptide of *POMC* and previous studies have indicated that variants in this domain probably alter POMC production and secretion, consequently influencing the development of obesity and type 2 diabetes in humans (Creemers et al., 2008; Mencarelli et al., 2012).

Cats heterozygous for the *POMC*:c.28G>C variant had a significantly higher BCS compared with cats homozygous for the reference allele. While the difference was not significant for %BF, the findings showed a similar trend, with heterozygous animals having a higher amount of body fat (median increase of 3%). Owing to the low number of animals homozygous for the alternative allele, it was not possible to assess the impact of this variant. While it appears that one of these animals has a significantly reduced body fat content, it is possible that this is a technical artefact caused by incorrect positioning or movement.

Given the small population studied and that no functional experiments were conducted to confirm causality, further studies are required to fully understand the possible impact of this newly described variant.

KEYWORDS
body weight, disorder, feline, genetic, genetic marker

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

POMC genotyes are available through the European Variant Archive under accession number: PRJEB63402. All data are further available in Tables S1 and S2.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.