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Implication of the G145C polymorphism (rs713598) of the *TAS2r38* gene on food consumption by Brazilian older women

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ABSTRACT

To evaluate the capacity to perceive bitter taste in a sample of the elderly population of the Brazilian Federal District, and to investigate its association with the consumption profile of distinct food groups. A total of 255 female outpatients aged 60 years or older took part in this cross-sectional study. The following data were determined for all the volunteers: alimentary frequency by clinical dieticians; genotyping of the G145C polymorphism in the *TAS2r38* gene; cognitive status; sensorial (visual and hearing) acuity and drugs related to ageusia or dysgeusia. Sensitivity to bitter taste was assessed using phenylthiocarbamide (PTC) in a subset. Non-parametric tests confirmed the remarkable effect of the C allele in determining sensitivity to PTC ($p < 0.001$). C allele carriers displayed diminished consumption of type B vegetables as well as of some vegetables generally recognized as bitter: arugula ($p = 0.044$) and chard ($p = 0.006$). No associations were observed for the remaining food classes. The present findings suggest that the G145C genetic variation in the *TAS2r38* gene modestly influenced food consumption habits of Brazilian older women. Nonetheless, the results do not rule out possible effects of past experiences on choices of elderly individuals.

1. Introduction

Food preferences and habits are influenced by environmental factors, cultural factors, availability and also by genetic characteristics. Food choice is determined by nutritional and physiological necessities, as well as a variety of sociocultural influences, such as personal eating experiences, perception of the health benefits that food can confer, cost, convenience and other properties (Birch, 1999).

However, taste is clearly the most important factor in the determination of alimentary preferences or aversions (Glanz et al., 1998). Sensitivity to phenylcarbamide (PTC) and its equivalent, 6-n-propylthiouracil (PROP), both containing thiocyanate that is responsible for the bitter taste characteristic (Tepper, 1998; Kim et al., 2004), is related to rejection of foods that possess this flavor such as vegetables of the *Brassica* genus, including kale, broccoli and turnip, among others, in addition to beer, cured cheeses, wine and tea. Individuals who are not sensitive to these substances have a more extensive range of food selections (Birch, 1999; El-Sohemy et al., 2007). Studies show that approximately 75% of humans are able to perceive the bitter taste associated with these compounds, while others do not show any perception or need high concentrations of these compounds to experience the bitter taste (Guo and Reed, 2001).

Genetic variation of the different types of receptors to sweet, salty, sour, bitter and savory tastes can explain at least part of these phenotypes. A number of single nucleotide polymorphisms (SNPs) in genes that code for *taste receptors* (*TAS*), with emphasis on the *TAS2r* gene family located on chromosomes 5, 7 and 12 (Kim et al., 2003), have been identified and implicated in differential sensitivity to bitter taste among individuals (Timpson et al., 2007; Garcia-Bailo et al., 2009). For perception of the bitterness of

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PROP, for instance, the peak association relies with the G145C SNP (rs713598) of the *TAS2r38* gene, which accounts for 45.9% of the trait variance (Reed et al., 2010).

Studies on food preferences are usually performed on children and/or young adults due to the fact that sensitivity in this age range tends to render a more clear aversion/acceptance behavioral pattern due to the absence of major cultural and psychosomatic influences in the consumption, and therefore with genetic variation exerting the strongest influence on food preferences (Mennella et al., 2005). In line, association between *TAS2r38* genotypes and food preferences has been found among younger strata, but no associations have been reported among older adults (Navarro-Allende et al., 2008). In contrast with younger populations, it is believed that acquired factors in the elderly play a stronger role than just the genotypes. This may derive from the fact that aging takes place on subjects with physiological and sociocultural backgrounds that may influence the perception of flavor and account for more vehement food consumption habits that do not only depend on the genetically-driven perception of taste.

In this scenario, one should remember that flavor sensation may diminish throughout life either by a reduction in the number of taste receptors (Cowart et al., 1994), or by the continuous use of medicines (Tomita and Yoshikawa, 2002). In line, studies show that the elderly tend to increase the variety of bitter tasting food in their diet (Lindgren, 1962). All in all, Brazil lacks studies concerning these determinants of the nutritional habits of its elderly population, and the influences and outcomes that surround this subject (Nobrega et al., 2009, in press), regardless whether genetic or environmental. Hence, this study will analyze the relationship between the classic G145C variation of the *TAS2r38* gene (coding for the synonymous A49P polymorphic variants, respectively) with sensitivity to bitter taste and with the pattern of food consumption in this age stratum. Because factors such as age strata, sex, cognitive status, sensorial acuity, use of medication and socio-demographic characteristics may affect sensitivity to bitter taste and the pattern of food consumption, this report poses a contribution by standardizing these variables in our sample.

2. Methodology

2.1. Study design

This is a cross-sectional, blinded study, with a convenience sample of female outpatients aged 60 or over recruited from the low income outskirts of the Brazilian Federal District to be regularly taken care of by the Interdisciplinary Elderly Health Promotion Project (EHPP) which is held by both the Graduation Program in Gerontology and the Geriatric Medical Service of the Catholic University of Brasilia. Additional characterization of subjects is presented elsewhere (Bortolon et al., 2008; Paula et al., 2010).

Subjects had a blood sample taken for DNA-extraction and attended a consultation with EHPP's nutritionists for the collection of feeding frequency and cognitive status, along an interview with a clinical pharmacist for evaluation of current medication. Following that, a subset was evaluated for visual and auditory gradation, and taste sensitivity. This was a blinded study since the clinical staff was not aware of the genotype/phenotype of each subject at the moment of food/pharmaceutical/cognitive data collection and taste evaluation, whereas the technician at the laboratory had no access to these data prior or after genotyping. This project was approved by the institutional Ethics Committee.

2.2. Analysis of the TAS2r38 gene

DNA samples were isolated using a routine protocol from peripheral blood leukocytes collected in Vacutainer tubes containing EDTA. The G145C transversion in the *TAS2r38* gene (rs713598) was genotyped using an original polymerase chain reaction (PCR)-based protocol developed for this study. Briefly, the complete messenger RNA sequence of the gene was obtained as deposited in the electronic genomic bank *GenBank* under the reference number NM_176817. Based on this sequence, forward (TAS-F1: 5′AAAGGTCCTGGCTTGGAACG3′) and reverse primers (TAS-R1: 5′AACGGATGAGCTTGGAGCAG3′) were designed to amplify a fragment of 482 bp which contains the polymorphic point of interest. Each reaction (25 μl) was composed of 100 ng of DNA, 10 mM Tris–HCl, pH 9.2, 25 mM KCl, 1.5 mM $MgCl_2$, 0.2 μM of each primer, 0.2 μM of each dNTP, 0.01 mg/ml of ovalbumine and 0.1 unit of Taq Polymerase (Phoneutria, Minas Gerais, Brazil).

The amplification program was composed of an initial denaturation step (hot start) at 94 °C for 2 min, followed by 36 cycles of: denaturation step at 94 °C for 40 s; annealing step at 64 °C for 45 s; extension step 72 °C for 50 s; polished by a final extension step at 72 °C for 5 min. The PCR products were submitted to electrophoresis in 1.6% agarose gel to verify product amplification and exhaustion of primers. Once primer exhaustion was visually verified, the identification of the polymorphic point was performed by direct sequencing of each amplification product using a 3130 DNA Analyzer system (Foster City, CA, USA) following the manufacturer's reagents and recommendations (Applied Biosystems). The primers used for sequencing were the same used in PCRs. Each electropherogram (sense and/or anti-sense) was rendered successful only when mild noise was observed using the Staden package of softwares for genomic analysis (MRC, Cambridge, UK) and allowed coincident identification of the polymorphic site by two independent readers after visual inspection. Genotyping success rate scored at 100%.

2.3. Evaluation of food consumption

The evaluation of foods consumed was obtained by means of the analytical method for alimentary frequency assessment whereby consumption habits and preference for certain types of food were detected (Pao et al., 1985). This frequency was collected through a nutritional survey and complemented by a questionnaire about food preferences carried out at the end of the sensitivity test. To improve reliability of the data, each patient was assessed with a validated Brazilian Portuguese version of the Mini-Mental State Examination (Folstein et al., 1975). Functionally illiterate subjects were excluded from the study, according to the following cut-off points: score 11/30 for illiteracy, 17/30 for individuals with 7 years of formal education, and 25/30 for individuals with 8 or more years of formal education (Castro-Costa et al., 2008).

The data collected with the alimentary frequency questionnaire was entered into an electronic spreadsheet and sorted into groups of foods: dairy products, legumes, meat, cereal, sugars, oils, fruits and vegetables. Following that, vegetables were sorted according to a classification system (Ornellas, 2006; Borjes et al., 2010) based on the combined criteria of structural properties and nutritional quality expressed by carbohydrate content that roughly applies as follows: type A for 5%, type B for 10% and type C for 20%. Main type A vegetables found were land cress, lettuce, chicory, arugula (rocket), chard (Swiss chard), spinach, arrowleaf elephant ear, onion, eggplant, broccoli, endive, kale, gilo (scarlet eggplant) and cabbage. Type B vegetables were represented by pumpkin, beetroot, carrot, chayote (pear squash), peppers, okra and green beans. At last, the type C vegetables included the sweet potato, the English potato and arracacha, winged yam, common yam and manioc. The ingestion frequency of the respective groups was classified as daily, weekly and monthly consumption, or non-consumption, in addition to the amount of consumption within each category.

After the above nutritional and clinical data were fully supplied, the following cases were excluded from analysis: those who did not have their feeding frequency collected; those who used estrogen replacement therapy or pharmaceutical products known to cause ageusia or dysgeusia; those who had inadequate cognition status.

2.4. Gradation evaluation

Prior to taste evaluation, gradation tests were performed to assess the ability of the subject to correctly judge phenotype intensity using human sensory functions only, namely vision and hearing.

In the first test, the subject listened to a sequence containing seven repetitions of sound (the note "La", produced using a normal tuning fork), three consecutive times. The sound was reproduced in increasing volume. After listening to the complete series, the sound was reproduced at one selected volume. The subject had to tell the interviewer what she thought the intensity of the sound was on an analogical scale of one to seven. In the second test, the subject was shown a series of seven shades of blue ranging from light to dark. This was repeated three consecutive times. One of the seven shades, selected at random, was then shown to the subject who had to tell the interviewer what shade she thought it was on an analogical scale of one to seven.

The acceptance criteria adopted was the correct indication of sound volume or shade of blue by the subject. The immediately adjacent intensity (either up or down) was also considered to be acceptable. Those older women who showed impaired visual or auditory acuities were ruled out from further analysis.

2.5. Taste evaluation

A subset of the sample was evaluated in relation to bitter taste sensitivity. PTC, sodium benzoate and thiourea were the chemicals selected for this purpose. These compounds are innocuous and do not pose any health risks. Each subject received a piece of filter paper separately impregnated with each of the aforementioned chemicals (Carolina Supplies, North Carolina, USA), and was instructed to place each filter paper directly on the tip of her tongue in the predetermined sequence, and to feel and interpret its taste. The order in which the filter paper were supplied was as follows: control (no chemical); test one with PTC; test two with thiourea; test three with sodium benzoate. The control had the aim of familiarizing the candidate with the physical properties of the filter paper only so to avoid confusion with the taste of the test chemical. Prior to, and between each tasting, the subject was asked to rinse her mouth out with distilled water for a total of 30 s. After tasting, the filter paper was discarded and the reaction of the subject recorded as well as a questionnaire documenting her preferences with regards to foods that present known or potential bitter taste. Subjects who declared sensitivity to each substance, regardless of intensity, were considered tasters. Those who had a cold at the time of the test or in the previous week and those who had eaten or used mouthwash were rescheduled to a minimum time span of one-week later.

2.6. Statistical analyses

Data analysis was performed using Microsoft Excel (Microsoft® Office® 2000) and SPSS® software for Windows® (version 10.0) using a non-parametric approach based on the chi-square test and the Fisher exact test. When applicable, statistical analysis was performed by reliable estimation of intervals for each determined ratio, assuming a confidence level of 95% in each estimate ($z = 1.96$).

3. Results

From our databank of 305 female outpatients, fifty subjects were ruled out based on the exclusion criteria, leaving a final sample size of 255 subjects. Exclusions were as follows: three for use of drugs related to ageusia or disgeusia (phenylbutazone, allopurinol or penicillamine); seven for use of estrogen replacement medication; nine for insufficient cognition; 15 for incomplete nutritional data; 16 for failing the acuity tests. Of the 255 subjects enrolled, a subset of 142 underwent the PTC, thiourea and sodium benzoate sensitivity tests to confirm the specificity of the *TAS2r38* gene for the sensitivity to PTC, and to demonstrate the functional characteristic of the G145C polymorphism. Initially, it was observed, that under the conditions of this study, 64.0% ($n = 91$) were sensitive to PTC, while 98.5% ($n = 140$) presented sensitivity to thiourea and 100% were sensitive to sodium benzoate. Non-parametric analysis of the data confirmed a marked association of the genotype with sensitivity to PTC, since 90% of the CC genotype and 79% of the heterozygotes presented detectable sensitivity, while only 13% of the G homozygotes showed sensitivity to the substance ($p < 0.001$). No differential sensitivity to sodium benzoate or thiourea could be attributed to the TASr38 genotypes.

Of the whole-group ($n = 255$) alimentary frequency analysis, one could notice the expected predominance of usual Brazilian food stuffs in the habitual diet of the older women. The most consumed foods were: rice (consumed by 99.6% of the sample), fruit (98.4%), type A vegetables (98.0%), beans (98.0%), type B vegetables (97.2%), type C vegetables (97.2%), poultry (94.5%), coffee (92.5%), salted biscuits (91.0%) and bovine meat (89.0%). Still consumed by a vast majority of the sample, albeit in a lesser proportion, were the following: pasta (82.0%), fish (80.7%), tea (78.8%), sugar (76.8%), cheese (74.9%), white bread (71.0%), sweets (68.2%), natural juice (65.0%), oil (61.6%), integral milk (60.4%), vinegar (60.7%), margarine (58.4%), soft drinks (55.2%) and sauces (54.1%). A minority of the elderly people studied consumed the following foods: pizza (45.4%), cream cheese (45.0%), sweeteners (44.3%), sweet biscuits (44.0%), mayonnaise (40.0%), honey (39.4%), processed juices (30.0%), butter (29.4%), integral bread (25.0%), jam (24.7%), semi-skimmed milk (23.9%), chocolate-flavored drinks (22.0%) and skimmed milk (5.4%).

The genotypes were distributed as follows in the sample: 23.5% ($n = 60$) were GG, 50.6% ($n = 129$) were of the GC genotype, and 25.9% ($n = 66$) were C homozygotes. The frequency of the genotypes in the sample was found to meet the Hardy–Weinberg distribution ($p > 0.05$). When the distribution of consumers and non-consumers of the food items specified above was evaluated across *TAS2r38* genotypes, consumers of type B vegetables were found to be significantly more frequent among G homozygotes ($p = 0.03$). In the subset of PTC-tested subjects, a comparable higher frequency of consumers of type B vegetables was found among non-tasters ($p < 0.05$). These findings are strengthened by the analysis that considered the frequency with which these foods were consumed, whereby the highest frequency (daily) was also observed among GG individuals (Table 1). G homozygotes, as mostly insensitivity to the bitter taste of PTC, have shown to be consumers of type B vegetables more frequently than C carriers. A comparable association was not seen for the consumption frequency of types A and C vegetables, although a trend was observed for type A vegetables.

Given these results, we proceeded to a detailed evaluation of consumption profile taking into account foods with known bitter taste, namely: radish, spinach, broccoli, chard, chicory and arugula (Drewnowski et al., 2001). In this analysis, we added typical Brazilian foods with proven bitter taste: gilo, west Indian gherkin (maxixe) and Indian coleus. Following this, we proceeded by grouping the genotypes according to a recessive (CC *vs.* GG + GC) or

Table 1
Frequency of consumption of types A, B and C vegetables across genotypes of the *TAS2r38* gene.

Vegetables	Genotype	Frequency				χ^2	p
		Daily *n* (%)	Weekly *n* (%)	Monthly *n* (%)	No consumption *n* (%)		
Type A	CC	42 (63.4)	22 (33.4)	2 (3.2)	0 (0)		
	GC	91 (70.5)	32 (24.8)	2 (1.5)	4 (3.2)	11.70	0.069*
	GG	51 (85.0)	8 (13.4)	1 (1.6)	0 (0)		
Type B	CC	36 (55)	25 (37.5)	5 (7.5)	0 (0)		
	GC	77 (59.7)	40 (31.0)	5 (3.9)	7 (5.4)	14.68	0.023*
	GG	44 (73.4)	16 (26.6)	0 (0)	0 (0)		
Type C	CC	34 (51.6)	29 (43.9)	3 (4.5)	0 (0)		
	GC	55 (42.6)	54 (41.8)	15 (11.7)	5 (3.9)	10.01	0.124*
	GG	36 (60.0)	17 (28.3)	5 (8.3)	2 (3.4)		

* Fisher Test. Degree of freedom = 3.

Table 2
Distribution of consumers and non-consumers of bitter taste vegetables across genotypes of the *TAS2r38* gene according to a recessive model of analysis.

Vegetable	Genotype	Consumption		χ^2	p
		Consumers *n* (%)	Non-consumers *n* (%)		
Radish	CC	15 (50.0)	15 (50.0)	1.539	0.215**
	GC + GG	70 (62.5)	42 (37.5)		
Spinach	CC	22 (73.3)	8 (26.7)	0.090	0.764**
	GC + GG	79 (70.5)	33 (29.5)		
Gilo	CC	27 (90.0)	3 (10.0)	0.692	0.405*
	GC + GG	94 (83.9)	18 (16.1)		
Broccoli	CC	26 (86.7)	4 (13.3)	1.172	0.280*
	GC + GG	104 (92.8)	8 (7.2)		
West Indian Gherkin	CC	28 (93.3)	2 (6.7)	4.429	0.041*
	GC + GG	85 (75.9)	27 (24.1)		
Indian Coleus	CC	19 (63.3)	11 (36.7)	0.030	0.863**
	GC + GG	69 (61.6)	43 (38.4)		
Chard	CC	9 (30.0)	21 (70.0)	7.453	0.006**
	GC + GG	65 (58.1)	47 (41.9)		
Common Chicory	CC	17 (56.7)	13 (43.3)	1.311	0.252**
	GC + GG	76 (67.8)	36 (32.2)		
Arugula	CC	15 (50.0)	15 (50.0)	4.040	0.044**
	GC + GG	78 (69.6)	34 (30.4)		

* Fisher Test.
** Chi-square test. Degree of freedom = 1.

a dominant model (CC + GC *vs.* GG) to verify whether or not different *TAS2r38* genotypes exerted any influence over the consumption frequency of these foods. In the so-called model of recessive effect, the habit of consuming vegetables such as chard and arugula was found to be significantly rarer within C homozygotes than in carriers of the G allele (Table 2). Not contrarily, analysis according to a dominant model found borderline, non-significant associations ($0.05 < p < 0.07$) suggesting less frequent consumption of these same food items among C carriers. When the distribution of consumers and non-consumers of these foods was evaluated across the subset of tasters and non-tasters of PTC, consumers of chard ($p = 0.02$), but not arugula ($p = 0.09$), were found to be less frequent among tasters.

With regard to the West Indian gherkin consumption analysis, the results differed from those previously mentioned. The group of C homozygotes showed a greater ratio of consumers when compared to G carriers, suggesting that the CC genotype may act as a trigger to consumption of this vegetable.

4. Discussion

The relationship between taste sensitivity and genotype of the *TAS2r38* gene was clearly observed in this study. Carriers of the C allele, predominantly homozygotes, showed sensitivity to PTC, whereas the carriers of the G allele did not demonstrate the ability to taste the flavor of this substance. This genetic finding is commonplace, and has even been used as evidence in paternity tests before molecular probes were available (Cardullo and Holt, 1951). Studies also show that women tend to be more sensitive to PTC than men, since sensitivity seems to be greatly influenced by sexual hormones (Guo and Reed, 2001). Being conducted only with postmenopausal women devoid of estrogen replacement medication, our study tends to pose a contribution by avoiding gender-related and hormonal influences in our results.

According to the literature, the foods with greater rejection indices within PTC-sensitive individuals are vegetables such as broccoli, kale, cauliflower, chicory and rhubarb; drinks such as coffee, beer and green teas; soya-derived foods and cured cheeses (Anliker et al., 1991; Duffy and Bartoshuk, 2000; Kaminski et al., 2000; Keller et al., 2002; Turnbull and Matisoo-Smith, 2002; Prescott et al., 2004). In our study, these results were not reproduced. Moreover, the ingestion frequency of several classes of food such as cereals, pasta, meat, dairy products, sweets, legumes, fruit, fat, bread, among others, was found not to be influenced by the genetically determined sensitivity to PTC, with the exception of vegetables. In this last case, it was observed that the consumption frequency of these vegetables, especially those commonly known to have a bitter taste, was significantly influenced by the genotype, since sensitive individuals reduced their consumption of these foods to zero. In our conditions, the

leafy vegetables arugula and chard were the bitter foods with the lowest threshold for tolerance by PTC-tasters. These results reassure that the C allele, responsible for PTC sensitivity, contributed to the aversion to bitter foods among the elderly women. The observation of significant results mostly when C homozygotes were analyzed apart from heterozygotes (recessive model) may reflect a dose-dependent allelic effect on the phenotype, in conformity with the observed increase in proportion of PTC-tasters from G to C homozygotes across genotypes.

Despite the close relationship between PTC sensitivity and the aversion to bitter foods, this phenomenon did not occur in a generalized manner since no significant association was detected between consumption and sensitivity on what concerns foods such as gilo, Indian coleus, broccoli, chicory and radish. There is evidence that elderly people tend to exhibit a more diversified diet patterns than younger individuals (Lindgren, 1962; Pao et al., 1982). This fact could be the result of cultural and life experiences as well as of learning healthcare practices involving eating habits, thereby masking the effect of the genotype in the behavior. Studies also show that individuals who were PTC-sensitive at birth may experience decreased sensitivity as a result of aging, physiological alterations or even illnesses (Stevens et al., 1995; Bartoshuk et al., 1996). These in fact may account for the counterintuitive West Indian gherkin consumption outcome. But this discussion has not been settled, and studies as the one performed on ingestion of *Brassica* genus vegetables (cabbage, turnip, broccoli, among others) by PTC-sensitive elderly women found that sensitivity was maintained, even in very old individuals, with no influence on vegetable consumption (Niewind et al., 1988).

Aversion to bitter taste vegetables based on common genetic variations demands concern as these vegetables are important sources of vitamins, antioxidants and minerals. It is also well documented that they provide bitter taste phytochemicals, chemoprotectors, lignans and phytoestrogens with reputed protective properties for several types of disease, including cancer (Stoner et al., 1991; Drewnowski et al., 2001). In addition, they are rich in fiber and low in calories (Philippi, 2002), which makes them food options to facilitate body fat loss and allow maintenance of a balanced diet. Our data are in favor of efforts for flavoring foods to elderly persons so to avoid bitter-taste associated dislikes, along with increase appreciation and healthy intake.

Despite the standardization of subjects in terms of gender, cognitive/sensorial performance, and socio-demographic characteristics, the present study has limitations. Any apparent disparity in results might be attributed at least in part to confounding factors inherent to the Brazilian scenario, such as the remarkable multiethnic origin of the population (Nobrega et al., 2009) accounting to possible variation in dietary habits (e.g. supplementation) not investigated here. Moreover, eating behavior in Brazil is more strongly influenced by socioeconomic status other than ethnic background (Fisberg et al., 2006). In this respect, our assessments took place with a rather homogeneous, low income elderly sample, what tends to attenuate disparities related to socioeconomic status. But it is important to acknowledge that results presented herein may not be extended to other population strata. Nonetheless, it is our perception that the observed food rejections cannot be justified by such interferences as these vegetables are not only accessible in economic terms, but also widely available in grocery establishments in the Brazilian Federal District.

It can be concluded that a genetic variation relative to the TASr38 gene was found to influence, albeit in a discrete way, the pattern of food consumption of the Brazilian elderly population, having observed that the C allele carriers, responsible for conferring sensitivity to the PTC taste, exhibited reduced consumption of specific bitter taste foods. The genetic influence on food preferences, although more clearly seen in children and young individuals, was observed for the elderly participants. The observation that the eating behavior of senior citizens are affected by biological factors does not rule out considerations regarding sociocultural and psychological factors that are known to influence equally, if not to a greater extent, food choices of the elderly.

Conflict of interest statement

None.

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