



# Environmental and Genetic Contributions to Indicators of Oral Malodor in Twins

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This study aimed to: (1) determine concordance rates of self-reported and subjectively determined indicators of oral malodor in twins; (2) determine the relative contributions of genetic and environmental factors to levels of volatile sulfur compounds (VSCs) in intraoral and exhaled breath. Fifty-one twin pairs participated in the study. Measurements of VSCs were obtained by a halimeter. The presence of tongue coatings was determined and twins filled out a 32-item questionnaire on oral malodor indicators independently of one another. Estimates of heritability ( $h^2$ ) for halimeter measurements were computed by SOLAR. The concordance rates for the presence of tongue coating among identical and fraternal twins were 67% and 11%, respectively. In the 10 most informative items, 70% exhibited higher concordance rates for identical than for fraternal twins. Of particular interest were the differences in concordance rates for dry mouth, sinus infection and unusual sweating. The  $h^2$  for intraoral breath was  $0.28 \pm 0.17$  (NS), whereas the  $h^2$  for exhaled breath was  $0.50 \pm 0.20$  ( $p = .0207$ ). The concordance rates of tongue coatings and malodor indicators were higher in identical twins than in fraternal twins. Intraoral breath VSC values were primarily attributable to environmental factors, whereas exhaled breath VSC values were partially explained by genetic factors.

**Keywords:** twins, malodor, heritability

Oral malodor affects up to a third of the general population and carries significant social and psychological ramifications (Frexinos et al., 1998; Miyazaki et al., 1995; Loesche & Kazor, 2002). Between 80% and 90% of oral malodor originates from the oral cavity, with other sources of oral malodor arising from the digestive (gastrointestinal tract) and respiratory (sinuses, lungs) pathways as well as from ingested foods (de Boever & Loesche, 1995; Waler, 1997). Oral production of malodorous substances is often associated with microbial metabolic by-products present on or in dental and mucosal surfaces, periodontal pockets and the tongue dorsum. The primary source of microbial metabolic by-products is residual food adhering to oral surfaces and, to a lesser extent, fermentation of proteins found in oral fluids and in lysed and desquamated cells (Loesche & Kazor, 2002).

Oral samples such as saliva, dental plaque biofilms and tongue coatings can produce volatile sulfur compounds (VSC), and they are primarily responsible for the produc-

tion of methyl-mercaptan and hydrogen sulfide (de Boever & Loesche, 1995), as well as short chain fatty acids and cadaverine (Raven et al., 1996), among other odorous compounds. Gram-negative anaerobic bacteria are primarily responsible for the production of metabolic by-products present in the tongue, saliva and dental plaque biofilms (de Boever et al., 1994; Hartely et al., 1996; Morita & Wang, 2001).

It is apparent that environmental factors present in the oral domain significantly contribute to the production of oral malodor. To date, the relative contribution of genetic factors to parameters related to oral malodor has not been

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investigated. The twin study model permits preliminary assessment of the relative contributions of genetic and environmental factors to a characteristic of interest (in this case indicators of oral malodor). If the correlations of parameters to be studied are higher in MZ (monozygotic) twins (100% genetically similar) and lower in DZ (dizygotic) twins (50% genetically similar), this suggests a genetic contribution to the parameters in question. Determining the relative contributions of genetic and environmental factors is essential to an etiological analysis. Twin studies are among the best models for quantifying these effects.

Anecdotal evidence suggests that a number of self-reported and subjectively determined indicators may uncover factors related to oral malodor. These indicators can be obtained by structured questionnaires and visual examinations that are often used in halitosis clinics (Roldan et al., 2005). Concordance rates obtained from MZ and DZ twins point to similarities and dissimilarities between twin groups, indicating genetic and environmental contributions to the parameters measured.

The purpose of this study was twofold: (1) to determine concordance rates of self-reported and subjectively determined indicators of oral malodor in MZ and DZ twins; and (2) to determine heritability estimates based on measurements of VSCs of intraoral breath and exhaled breath by employing a halimeter that measures VSC levels.

## Materials and Methods

### Participants

Fifty-one twin pairs (31 MZ and 20 DZ twins, total  $n = 102$ ) between 12 and 21 years of age participated in the study. The mean age of the MZ pairs was 14.6 years and that of the DZ pairs was 15.9 years. The zygosity of twin pairs was determined by genotyping highly polymorphic markers (Corby et al., 2007). There were 16 female and 15 male MZ pairs, and 3 male/male, 4 female/female and 13 male/female DZ pairs. Participants that were in good general health and without any disease or conditions that could be expected to interfere with the study procedures were enrolled in the study. The twins signed an informed consent form that was approved by two institutional review boards. The study took place in the city of Montes Claros, Minas Gerais, Brazil.

### Exclusion Criteria

Criteria for not enrolling twins included at least one member of the twin pair presenting with one or more of the following conditions: (1) any condition requiring the need for antibiotic medication prior to dental procedures; (2) self-reported pregnancy; (3) mild to severe periodontal disease; (4) active treatment for periodontitis (current or within the past 6 months); (5) presence of any open dental caries lesions; and (6) use of systemic antibiotics within the past 3 months.

### Tongue Coating and Self-Reported Oral Malodor Indicators

Dental examiners determined the presence or absence of tongue coating (bacterial mat) in twins. The original index was proposed by Winkel and colleagues (Winkle et al., 2003) and was slightly modified by dividing the dorsum of the tongue into three sections: a posterior, a middle and an anterior part. The presence of tongue coating was recorded for each of these sections, provided the coating covered more than 1/3 of each section. If at least two of three sections conformed to these guidelines, then the possible scores were 1 (the presence of tongue coating) or 0 (no coating).

Independently of each other, twin pair members filled out a 32-item questionnaire that included a number of potential oral malodor indicators (Tarzia, 2000). We elected to determine concordance rates on items that had at least a prevalence of 10% in MZ and DZ twins combined (range of prevalence rates = 10% to 80%). These included: a — alterations in taste perception; b — unusual tiredness; c — self-perception of bad breath; d — habitual chewing of gum; e — habitual candy consumption; f — mouth feels dry; g — sinus infection; h — unusual sweating; I — strong foot malodor; and j — daily, frequent water consumption (Table 1).

### Measurement of Volatile Sulfur Compounds

A chair-side portable instrument (Halimeter® (Interscan Corp., Chatsworth, CA) was used to measure the levels of VSCs in intraoral (ambient) breath (Rosenberg et al., 1991) and exhaled nasal breath (Monteiro-Amado et al., 2005). For the intraoral measurements patients were asked to maintain their mouths closed for 3 minutes. After 3 minutes a plastic straw attached to the halimeter was inserted into the subject's mouth and the subjects were asked to breathe through their noses during the measurements. For the exhaled (expired) air, measurements of nasal values were obtained by inserting another plastic straw 1 cm inside each nostril until peak values were achieved.

### Statistical Analysis

Concordance rates were employed to determine the presence of the same trait in both members of a pair of twins. Concordance rates exemplify the probability that a pair of individuals will both have a certain characteristic given that one member of the pair has the characteristic. For example, twins are concordant when both have or both lack a given trait. In this report, we limit the description of concordance rates to those based on the presence of a given trait.

Estimates of heritability ( $h^2 = V_G/V_P$ , where  $V_G$  is the additive genetic variance, and  $V_P$  is the total phenotypic variance) for halimeter measurements were obtained using the variance-component methodology implemented in the Sequential Oligogenic Linkage Analysis Routines (SOLAR) package, version 8.0, available at:

**TABLE 1**  
Self-Reported and Subjective Parameters Suggestive of Oral Malodor in Twins

Variables	Concordance Rates for Measured Parameters					
	MZ total	MZ Cn	MZ C (%)	DZ total	DZ Cn	DZ C (%)
Presence of tongue coating	27	18	67%	19	2	11%
<sup>a</sup> Alterations in taste perception	11	6	55%	16	10	62%
<sup>b</sup> Unusual tiredness	6	2	33%	8	2	25%
<sup>c</sup> Self-perception of bad breath	13	6	46%	6	2	33%
<sup>d</sup> Habitual gum chewing	47	36	77%	29	24	83%
<sup>e</sup> Habitual candy consumption	55	50	91%	34	28	82%
<sup>f</sup> Mouth feels dry	19	12	63%	12	2	17%
<sup>g</sup> Sinus infection	8	4	50%	6	0	0%
<sup>h</sup> Unusual sweating	8	6	75%	4	0	0%
<sup>i</sup> Strong foot malodor	5	2	40%	7	4	57%
<sup>j</sup> Increased daily water consumption	49	30	61%	29	16	55%

Note: MZ-monozygotic, DZ-dizygotic, Cn-count, C-concordance

<http://www.sfbr.org.solar/>. Variance component models of the type implemented in the SOLAR package do not simply take the difference in MZ and DZ twin correlations and multiply by 2.0 to get an estimate of heritability, but rather they model the covariation in twin-pair trait values as a function of kinship coefficient in a random effects linear model where one random effect (i.e., a variance component term) corresponds to the effect of kinship (the kinship coefficient for MZ twins is 1.0 and for DZ twins 0.5). This linear model also allows for covariate effects. The inclusion of covariates in the model and the unique way in which variance component models assess heritability can create differences in heritability estimates from those produced by overly simplistic estimates obtained from, for example, differences in MZ and DZ correlations unadjusted for covariate effects. Twin-based variance component models assume bivariate normality of the phenotype, and in our case, they were used to estimate narrow-sense heritability. Although differences in kinship that account for variation and covariation in a trait are likely to reflect genetic effects, there is the possibility that the differences in kinship that appear to influence variation and covariation in the trait reflect the effect of something merely correlated with kinship differences (e.g., some shared cultural or environmental effect). Thus, our estimates of heritability may be tapping into something more akin to 'familiality.' All models were adjusted for age and gender.

## Results

The concordance rates for the presence of tongue coating among MZ and DZ twins were 67% and 11%, respectively (Table 1). For the item questionnaire, concordance rates for each item are presented for MZ and DZ twins respectively: a-55%, 62%; b-33%, 25%; c-46%, 33%; d-77%, 83%; e-91%, 82%; f-63%, 17%; g-50%, 0%; h-75%, 0%; i-40%, 57%; j-61%, 55% (Table 1). Of the ten responses that were reported, 70% (7 of 10) exhibited higher concordance rates for MZ than for DZ twins. Of particular interest were the marked differences in concordance rates for dry mouth, sinus infection and unusual sweating.

The heritability for intraoral breath was  $0.28 \pm 0.17$  (NS), whereas for exhaled breath it was  $0.50 \pm 0.20$  ( $p = .0207$ ) (Table 2). This means that the variation in intraoral breath values was essentially modulated by the environment and that variation (50%) in exhaled breath values had a significant genetic contribution.

## Discussion

The current study is limited by its sample size and the validity of the questionnaires that were employed in the analyses. The trends observed in the results for concordance rates and heritability estimates of indicators of oral malodor nevertheless merit discussion. To our knowledge, this is the first report in the literature on the contributions of genetic and environmental factors to indicators of oral malodor that employed the twin study model.

**TABLE 2**  
Heritability Estimates for VSC Levels

Variable	Heritability ( $h^2$ )*	S.E.	P value	n
Intra-oral Air	0.28	0.17	NS	102
Exhaled Breath	0.50	0.20	$p = .0207$	102

Note: \*Adjusted for age and gender

The presence of a tongue coating is a common problem, and studies employing large cohorts have determined its prevalence to be about 45% (Quirynen et al., 2009). The papillary structure of the dorsum is a unique ecosystem in the oral cavity, offering a large surface area that favors the accumulation of oral debris and bacteria. The morphology of the dorsum of the tongue provides additional irregularities such as fissures, grooves and depapillated areas that may serve as retention areas that harbor bacteria (Krespi et al., 2006; Roldan et al., 2003a; Roldan et al., 2003b; van den Broek et al., 2008). The high concordance rates for tongue coating in MZ twins (67%) and low rates for DZ twins (11%; Table 1) suggest that some mechanism is operating that may govern the accumulation of bacterial mats on the tongue surface. Whether this is related to the MHC (major histocompatibility complex) and related HLA (human leukocyte antigen) genes involved in immunological modeling of microbial colonization of the oral cavity including the tongue surface remains to be determined.

The next set of indicators of oral malodor (self-reported) that had marked differences in concordance rates between MZ and DZ twins are related to gland and mucosal function, i.e., dry mouth, unusual sweating and sinus infection (Table 1). The reports of the mouth feeling dry and of unusual sweating are contrasting, but they indicate that body and salivary gland functions may be influenced by genetic factors. Similarly, the presence of sinus infections indicates a breakdown in mucosal defenses against bacteria and viruses, which may also be mediated by genetic factors. Indeed, animal studies suggest that genetic background is involved in responses to bacterial sinusitis (Kirtsreesakul et al., 2006).

In these sets of twins, we found that genetic factors make a significant contribution to the levels of VSCs in exhaled breath. The heritability estimates for intraoral air were  $0.28 \pm 0.17$  (NS), whereas for exhaled air they were  $0.50 \pm 0.20$  ( $p = .0207$ ). This means that the variation in intraoral breath values was essentially modulated by the environment (presumably volatile microbial by-products) as expected and, that variation (50%) in exhaled breath values had a significant genetic contribution. One can speculate that a number of non-oral factors may be under genetic influence, including the regulation of metabolic conditions involving enzymatic and transport pathways (Preti et al., 2006), leading to the systemic production of volatile compounds in exhaled air. This genetic contribution is population-specific because it focuses on latent omnibus genetic effects in population variation and not on effects of a specific identified genotype in individuals.

The quantification of trace gases present in exhaled air is timely because its potential for clinical diagnosis is being realized. Exhaled breath gases are often used to indicate early-stage metabolic diseases (Španěl et al., 2007). In our case, our population was ostensibly healthy, and gases detected by the halimeter may only be indicative of oral

malodor in a combination of intraoral breath and exhaled breath. Halimeter measurements have been shown to correlate with organoleptic rating scores, that is, with correlation coefficients ranging from  $r = 0.42$  to  $r = 0.64$  (Kazor et al., 2003). These correlations indicated that the VSCs measured by the halimeter account for only 18 to 41% of the organoleptic rating score, indicating that there are other important compounds, such as volatile fatty acids, cadaverine and other compounds present in exhaled breath that contribute to the organoleptic rating score but are not detected by the halimeter. This explains the anomalous finding that sometimes malodor can be detected by the examiner, but the VSC levels are in the low range (Hartley et al. 1996; Miyazaki et al., 1995). This further suggests that additional studies involving an increased number of compounds in intra-oral and exhaled (expired) breath should be evaluated in the twin study model as well as the inclusion of organoleptic measures to validate these findings.

Although our results are preliminary, taken together they suggest that studies in larger cohorts of twins should be conducted to dissect the relative contribution of genetic and environmental factors to indicators of oral malodor. In addition, the contributions of these factors to objective parameters of malodor (VSCs and other markers) should be further evaluated in larger cohorts of twins with subsequent genomic analyses.

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