Chem. Senses 32: 749–754, 2007

doi:10.1093/chemse/bjm042
Advance Access publication July 10, 2007

Twin Study of the Heritability of Recognition Thresholds for Sour and Salty Taste

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Abstract

Seventy-four pairs of monozygotic (identical) twins and 35 pairs of dizygotic (fraternal) twins provided recognition thresholds (modified Harris–Kalmus test) for the sourness of citric acid and the saltiness of sodium chloride during the Twins Days Festival in Twinsburg, OH. Variance components (ACE) models were applied to the data: total variation = additive genetic (A) + common environment (C) + nonshared environment (E). The best-fit model of variation in recognition thresholds for sourness included an additive genetic factor, accounting for 53% of the variance, but no common environment component. This level of heritability, on par with that of sensitivity to the bitter compounds 6-n-propylthiouracil and phenylthiocarbamide, strongly suggests that genetic factors play a larger role than shared environment in determining individual differences in recognition thresholds for sourness. In contrast, the best-fit model for saltiness recognition included a common environment component, accounting for 22% of the variance in thresholds, but no additive component. This result suggests that environment plays a larger role than genetics in determining individual differences in recognition thresholds for saltiness.

Key words: genetics, NaCl, perception, pH, variability

Introduction

We lack a firm understanding of human sour and salty taste transduction. Genetic analysis of variation in sensitivity might help elucidate transduction mechanisms, as it has for sweet, bitter, and umami taste (Kim et al. 2004; Mombaerts 2004; Breslin and Huang 2006). However, few studies have documented population or individual differences in human sour and salty taste abilities (Blakeslee and Salmon 1935; Odeigah and Obieze 1986; Hladik 1997). Even fewer studies have asked whether there are heritable genetic contributions to variability in sour and salt sensitivities.

One study examined detection thresholds for the sour compound hydrochloric acid (HCl) in both identical (monozygotic, or MZ) and fraternal (dizygotic, or DZ) twins (Kaplan et al. 1967). According to the logic of classic twin studies, MZ twins have nearly identical genomes, whereas DZ twins, like siblings born separately, share on average 50% of their genomes in common. Assuming that MZ and DZ twin pairs reared together experience similar degrees of shared environment, that is, same in utero experiences, same parents, same house, same culture, etc., better agreement between MZ than between DZ twins suggests a genetic contribution to the trait in question. Because MZ twins did not resemble each other more than DZ twins with respect to detection thresholds for HCl, Kaplan et al. (1967) were unable to demonstrate that absolute sensitivity to sour taste is a heritable trait.

Genetic contributions to salt preference and consumption have received long-standing attention in humans (e.g., Greene et al. 1975; Beauchamp et al. 1985) and in animal models (e.g., Lush 1991; Beauchamp and Fisher 1993; Bachmanov et al. 1996; Tordoff et al. forthcoming). Extant reports, including the single reported human twin study on detection thresholds for sodium chloride (NaCl), have failed to demonstrate that variation in salty taste perception is heritable (Beauchamp et al. 1985). To the contrary, studies suggest that one's history of sodium exposure can have a substantial impact on preference for, consumption of, and physiological processing of NaCl (Crystal and Bernstein 1995; Stein et al. 1996; Pittman and Contreras 2002). It is possible that genetic contributions to salty taste variability

are sufficiently low compared with environmental causes of variability that heritability of salty taste perception is difficult to detect.

However, the absolute detection thresholds used to assess heritability of sensitivity to HCl and NaCl may not have measured sensitivity to sour and salty taste per se. For example, as some have noted, acids might stimulate other modalities, such as astringency or other somatosensory systems, at concentrations below the threshold for conscious taste quality perception (Kim et al. 2004). Accordingly, absolute detection thresholds may not strongly correlate with suprathreshold sensitivity. The current study measured recognition thresholds for the sourness of citric acid (CA) and the saltiness of NaCl, that is, the minimum concentrations that subjects report taste sour or salty in human twin pairs.

Materials and methods

Participants

The sample included 74 MZ and 35 DZ pairs of twins. Experimenters recruited and tested participants at an annual convention of twins, Twins Days Festival, in Twinsburg, OH. Testing occurred at the 2003 and 2004 festivals in August. Each year yielded roughly half the total sample. Participants included 57 males and 161 females, with ages ranging from 14 to 72 years (mean 28.3 ± 16.2).

Zygosity

Zygosity was assessed in 3 ways: subjects reported zygosity, 2 experimenters rated their photographs for physical similarity, and a subset of twins were genotyped. Cells from the inner cheek were obtained with swabs, and genomic DNA was extracted following manufacturer's directions (Epicenter, Madison, WI). Genotyping was conducted using a commercial kit (AmpF1STR Profiler Plus Amplification KIT, Applied Biosystems, Foster City, CA) that analyzed 9 independent, highly polymorphic DNA markers, plus the amelogenin marker for sex. This test gives a probability less than 10^{-4} that a pair of DZ twins are concordant at all 9 markers (Nyholt 2006).

For all twin pairs who contributed recognition thresholds for sour and salty taste, self-reported zygosity matched the independent, investigator-rated zygosity coded from facial photographs. For the subset of subjects who were genotyped ($N = 86$), DNA-based zygosity matched self-reported zygosity in all cases.

Stimulus materials

Stimuli included CA and NaCl (Sigma-Aldrich, St. Louis, MO) dissolved in Millipore-filtered deionized water. Concentrations ranged from undetectable to clearly detectable for most people (6.10×10^{-4} to 5×10^{0} mM for CA and 3.05×10^{-2} to 2.50×10^{2} mM for NaCl) in 14, 2-fold steps. Stimuli were prepared less than 1 week in advance of the festival and stored under refrigeration in amber glass bottles until utilized. Blanks for the sorting task (see General procedure) consisted of Millipore-filtered deionized water, stored, and handled the same way. Subjects rinsed with bottled drinking water (Sam's Club), purchased in Twinsburg. All solutions assumed ambient air temperature, approximately 30 °C, 24 h prior to testing.

General procedure

The procedure described below constituted part of a larger battery of tests. Others included tests of sensitivity to sweet, bitter, and olfactory stimuli. After providing written consent on forms approved by the Office of Regulatory Affairs at the University of Pennsylvania, subjects donated DNA via buccal swabs, posed for a photograph, and scaled the intensity of some sucrose solutions. The initial psychophysical test helped ensure that subjects had a functioning sense of taste and were able to follow the directions. Subjects participated in as many tests as they wished, with no predetermined order. This report will not refer to the other tests conducted.

Twin siblings usually completed the test at the same time. One twin (selected at random) completed tests for CA first, the other completed tests for NaCl first. Twins were not seated immediately next to one another, and experimenters instructed subjects not to discuss tests in progress. Experimenters informed twins that they were receiving different stimuli. Recognition thresholds for sour and salty taste were measured via a modified Harris–Kalmus procedure (Harris and Kalmus 1949). Testing began with the lowest concentration. Subjects received a single, 10-ml sample in a plastic medicine cup. Subjects held the sample in their mouths for 5 s, expectorated, and attempted to identify the quality of the taste. Options included "sweet," "sour," "bitter," "salty," or "water." Next, subjects rinsed at least twice with bottled water before receiving the next higher concentration. Concentration ascended in this fashion until subjects identified the requisite quality, that is, "sour" for CA and "salty" for NaCl. We make no assumptions about whether these 2 qualities are "correct" labels, as there can be no correct labels for all subjects. Rather, we are determining the lowest concentration required for subjects to provide a particular response as a way of measuring perceptual variability.

After subjects reported the required quality, they completed a sorting task to ensure that they were experiencing a reliable taste sensation. Subjects received 3 samples at the concentration at which they identified the expected quality plus 3 blanks (Millipore-filtered deionized water). The samples were presented at the same time, in 1 of 4 random orders. Subjects knew that exactly 3 cups contained stimuli and were required to sort the cups into "tastes" and "waters." If they could correctly sort the 6 samples in 2 consecutive trials, testing ended and the concentration at which subjects correctly sorted the stimuli served as the estimate of recognition threshold. If a subject failed to sort correctly, the sorting task

was repeated at the next higher concentration. The concentration that first allowed successive, correct sorts served as estimate of quality recognition threshold. As a confirmation of subjects' quality responses, the concentration next higher than the determined recognition threshold was presented to ensure that subjects reported the same label; in all cases they did.

Statistical analyses

The difference in genetic relatedness between MZ and DZ pairs was used to partition total variation in taste recognition thresholds into subcomponents. In the standard (Cholesky) model selected, total variation is divided into additive genetic influences (A), shared environment (C), and nonshared environment (E) (Neale and Maes 2004). The A component is greater than 0 if MZ twins resemble one another more than do DZ twins and increases as the difference between the MZ and DZ correlations increases. The C component, which reflects the influence of being raised in the same environment, would increase if both the MZ and DZ correlations rose together. The E component, which represents both environmental influences unique to each sibling and experimental error, increases as the MZ and DZ correlations decrease.

Some models also include a D component, which represents nonadditive genetic influences. However, common environment and nonadditive genetic effects are confounded unless one can compare twin pairs reared apart to twin pairs reared together (Neale and Maes 2004). Because our sample included only pairs reared together (according to subject report), we could include either C or D in the model, but not both. To decide whether a D parameter should be modeled, one can compare MZ correlations to DZ correlations. If the MZ correlation is more than double the DZ correlation, this suggests nonadditive genetic influences that can include dominance and epistasis (Neale and Maes 2004). Because MZ correlations did not exceed DZ correlations by more than 2-fold (see Results), no D component was modeled.

As an additional consideration, the recognition thresholds for CA and NaCl were weakly correlated with each other (see Results). Thus, a bivariate model can be specified that utilizes the additional information gained from the cross-trait correlations to estimate common sources of variation between the phenotypes. In this fuller model, the A, C, and E components each have 2 factors. One factor is common to recognition thresholds for both compounds, and one factor is specific to recognition thresholds for only one compound. This fully saturated model, that is, an identified model with the maximum number of estimated parameters, was used as a baseline to which simpler (nested) models were compared. In this model, the covariates of age and gender were modeled as regressions or deviation effects on the mean.

Analyses were performed with the statistical program Mx, which uses maximum likelihood estimation to fit the variance components models (Neale et al. 2002). The program quantifies goodness of fit as twice the negative log-likelihood ($-2LL$) and compares nested models on the basis of this statistic. One can examine how $-2LL$ changes as one simplifies the fully saturated model by removing components. The fact that differences in $-2LL$ are distributed asymptotically as χ^2 allows one to assign P values to differences between models in goodness of fit. We adopted the standard criterion for significance of $P < 0.05$.

Data were screened for normality and outliers in all models. To establish regularity in sampling and measurement, and satisfaction of the assumptions of twin-studies designs, Mx assessed the homogeneity of means and variances as described in Hansen et al. (2006). The distribution of NaCl was sufficiently normal for analysis. The CA thresholds were square root transformed to account for a small positive skew. Outlying twin pairs were detected and excluded using the %p option in Mx (Hansen et al. 2006). No twins were identified as outliers at the assumptions testing stage, but one pair was detected in the bivariate model and excluded from analyses.

Results

Both thresholds had homogenous means, variances, and covariances, and there was no evidence for age and sex effects. The MZ and DZ twin correlations had overlapping 95% confidence intervals (CIs) for both traits with estimates for the CA correlations at 0.53 (0.37–0.66) and 0.27 (−0.31 to 0.60), respectively, with NaCl estimates at 0.24 (−0.01 to 0.44) and 0.21 (−0.08 to 0.46), respectively. The thresholds of CA and NaCl were weakly correlated with each other ($r = 0.10$).

As described in Statistical Analyses, a bivariate Cholesky model was specified containing A (additive genetic), C (common environmental), and E (unique environmental) variance components. The significance of each parameter in this model was assessed by dropping the parameter and observing the resulting change in model fit. The order in which the parameters were dropped was determined by examining the parameter magnitudes and by expectations from the observed DZ and MZ correlations. The parameters dropped were the shared (between both compounds) C component (change in $-2LL$ of 1.86, 2 df, $P = 0.39$), the NaCl-specific A component (change in $-2LL$ of 0.01, 1 df, $P = 0.92$), and the NaCl loading for the shared A (change in $-2LL$ of 1.24, 1 df, $P = 0.27$) and E (change in $-2LL$ of 0.59, 1 df, $P = 0.44$).

After all drops that had no significant impact on model fit were made, the final (most parsimonious) model suggested that additive genetic effects played a more important role than common environment in determining variation in CA thresholds but that common environment played a more important role than additive genetic effects in determining NaCl thresholds (Figure 1). This model included no significant shared variance components between CA thresholds and NaCl thresholds. The model included an A component,

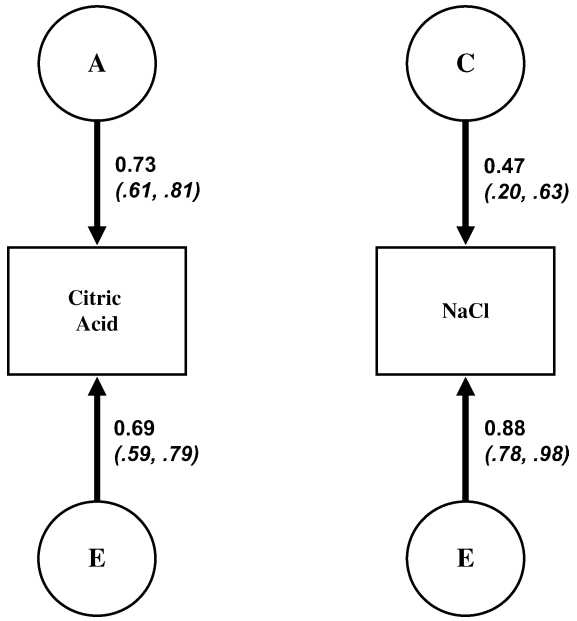

Figure 1 Standardized path diagram depicting the additive genetic (A), common environmental (C), and unique environmental (E) variation in recognition thresholds for CA and NaCl. Standardized path coefficients (which should be squared to obtain proportion of variance accounted for) are shown, with 95% CIs in parentheses.

but no C component, for CA. The additive genetic component accounted for 53% of variation in CA thresholds. Further, for NaCl, the model included a C component but no A component. The common environment component accounted for 22% of the variation in NaCl thresholds. Residual variation for both traits is due to unique environment and/or experimental error.

Discussion

Recognition thresholds for sour taste

Variance components modeling suggests that up to 50% of the variation in sensitivity to the sourness of CA is due to heritable genetic variation. An earlier twin study on detection thresholds for HCl found no evidence of heritability (Kaplan et al. 1967). However, as suggested in the Introduction, absolute detection may not correspond to detection of sourness per se. It is also possible that stimulus differences played some role. Organic acids like CA tend to taste sourer than inorganic acids like HCl at equal pH (Makhlouf and Blum 1972; Ganzevles and Kroeze 1987). Although sour taste transduction mechanisms may have evolved to detect organic acids in fruits, milk, and meats, there is as yet no evidence that organic and inorganic acids are transduced differently. CA, like many organic acids, is partially dissociating (weak), whereas HCl, like many inorganic acids, is fully dissociating (strong). For weak acids, protons passing into taste cells might be replaced by previously undissociated protons on the anion, effectively increasing the number of free protons sensed in the perireceptor environment. Other transformations of the stimulus in the oral mucosa are also possible.

Regardless, the current study has found heritability for sensitivity to sour taste nearly on par with that of sensitivity to the bitter compounds phenylthiocarbamide or 6-n-propylthiouracil (e.g., Martin 1975; Kim et al. 2003; Hansen et al. 2006). Heritable variation might arise at any stage of processing, but peripheral taste receptors are often responsible for individual differences (Bufe et al. 2005). The heritability of sour sensitivity suggests that a straightforward analysis of genes for candidate sour receptors or family-based linkage studies might prove fruitful as a first attempt at identifying the sources of genetic variation.

Research has suggested a number of candidates for sour taste receptors. The proton-sensitive polycystic kidney disease (PKD) transient receptor potential-related channel is expressed in taste receptor cells (Huang et al. 2006; Ishimaru et al. 2006; LopezJimenez et al. 2006). Moreover, when cells that express the relevant PKD channels are selectively ablated, animals are consequently blind to oral acids (Huang et al. 2006). Therefore, these PKD channels serve as markers for a subset of taste receptor cells that are necessary for encoding sour taste. If these channels also serve as the principal sour stimulus detectors, then it is possible that polymorphisms in PKD channel genes account for the heritable variation in sour taste sensitivity reported here. Other candidate sour taste receptors include acid-sensing ion channels, which are also expressed in taste receptor cells, proton-sensitive K channels, hyperpolarization-activated and cyclic nucleotide-gated ion channels, and, possibly, proton-sensitive G-protein–coupled receptors (Ugawa et al. 1998; Stevens et al. 2001; Richter et al. 2004; Breslin and Huang 2006). Analyses could determine whether polymorphisms in the genes that encode these proteins are associated with perceptual sour taste variability.

Other factors may play a role in individual differences in sensitivity to sourness. For example, individual differences in salivary flow rate leads to differences in the buffering capacity of the mouth (Christensen et al. 1987; Lugaz et al. 2005). Differences in buffering capacity in turn can lead to individual differences in sourness perception. Although the genes that determine salivary flow rate are unknown, studies could determine whether salivary flow rate is heritable.

Recognition thresholds for salty taste

In contrast to results for sour taste, recognition thresholds for salty taste had no significant heritable component. Recognition thresholds did show a strong component of shared environment, which may include the in utero environments of twins. Prior reports of salty taste heritability in humans have similarly demonstrated no heritable component of oral salt sensitivity (Beauchamp et al. 1985).

Perhaps the apparent importance of environment should not be surprising given that behavior toward salt is environmentally

labile. For example, children who suffered sodium imbalances as infants due to chloride depletion showed lasting alteration of preference for salty foods (Stein et al. 1996). Even in utero experiences of altered sodium regulation, such as sodium loss among pregnant women who vomit excessively from ''morning sickness,'' can have chronic effects on the subsequent salt responsiveness of the children (Crystal and Bernstein 1995; Crystal et al. 1999). Other research suggests that time-of-day (Irvin and Goetzl 1952) or even short-term exposure can have some (temporary) impact on salty taste (e.g., Ayya and Beauchamp 1992). It is unclear how preference and consumption relate to sensitivity to salty taste per se. However, it is clear that history of sodium exposure in animal models can have a substantial impact on the taste system at early levels of processing, that is, at receptor cells, fibers that innervate the taste buds, and the first taste relay in the brain (Stewart and Hill 1996; Hendricks et al. 2002; Pittman and Contreras 2002). Evolutionary forces may have shaped the human ability to recognize salty taste in such a way as to make it very responsive to differences in the environmental mineral and water supply or habitual diet (e.g., Jackson 1991).

In conclusion, our results strongly suggest that genetic influences play a more important role than shared environment for individual differences in sourness recognition thresholds and that shared environment plays a more important role than genetic influences for individual differences in salty recognition thresholds. Finally, we note that a larger and more optimally balanced MZ:DZ ratio sample could provide the power needed to better parse additive and common environment components and might find some influence of shared environment for sourness and some influence of genetic factors for saltiness.

Funding

National Institutes of Health (R01DC002995 to P.A.S.B., R01DC004698 to D.R.R., 5R03ES013969 to P.M.W.); Kraft Foods (Monell Term Chair in Chemosensory Psychophysics to P.M.W.); the Monell Foundation.

Acknowledgements

Gary K. Beauchamp and Michael Tordoff commented on earlier drafts of the manuscript. We thank the twins for participating. We also appreciate the cooperation of Sandy Miller and the other Twins Day conference organizers and the excellent technical assistance of Anilet Tharp, Christopher D. Tharp, and Thomas Canty.

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Accepted June 6, 2007