Chem. Senses 36: 9–17, 2011

doi:10.1093/chemse/bjq081
Advance Access publication September 27, 2010

Excretion and Perception of a Characteristic Odor in Urine after Asparagus Ingestion: a Psychophysical and Genetic Study

Marcia Levin Pelchat[1], Cathy Bykowski[2], Fujiko F. Duke[1] and Danielle R. Reed[1]

[1]Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104-3308, USA and [2]Department of Psychology, University of South Florida, 4202 East Fowler Avenue, Tampa, FL 33620-7200, USA

Correspondence to be sent to: Marcia Levin Pelchat, Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104-3308, USA. e-mail: pelchat@monell.org

Accepted July 26, 2010

Abstract

The urine of people who have recently eaten asparagus has a sulfurous odor, which is distinct and similar to cooked cabbage. Using a 2-alternative forced-choice procedure, we examined individual differences in both the production of the odorants and the perception of this asparagus odor in urine. We conclude that individual differences exist in both odorant production and odor perception. The biological basis for the inability to produce the metabolite in detectable quantities is unknown, but the inability to smell the odor is associated with a single nucleotide polymorphism (*rs4481887*) within a 50-gene cluster of olfactory receptors.

Key words: genetics, odorants, olfaction, psychophysics, specific anosmia

Introduction

Some people report that after eating asparagus, their urine has a sulfurous odor like cooked cabbage. For people who smell the odor, they know it to be a result of eating asparagus, whereas others appear to never smell the odor and are surprised to be asked about it. The unusual odor elicited by human urine after asparagus has been mentioned over the years; for instance, Benjamin Franklin noted that "a few stems of asparagus eaten shall give our urine a disagreeable odor" (Franklin and Japikse 2003), and Proust wrote more favorably that asparagus "as in a Shakespeare fairy-story transforms my chamber-pot into a flask of perfume" (Proust 1929). This phenomenon has also attracted the attention of scientists studying individual differences, and work in this area has been reviewed (Mitchell 2001).

There are 2 hypotheses about this phenomenon. The first hypothesis is that there is a polymorphism in production of the odorant, such that some people excrete the odorant but others do not (Allison and McWhirter 1956; Mitchell et al. 1987). Production of an unusual odorant is a cardinal feature of several inborn errors of metabolism, for instance, trimethylaminuria (also known as fish-odor syndrome), which results from a buildup of trimethylamine derived from choline metabolism (Humbert et al. 1970). One way to understand whether there are individual differences in asparagus odor production is to identify the odorant and measure it in urine; however, there is no convergence on specific compounds from the studies conducted to date (Table 1). The second hypothesis is that everyone produces the odorant, but individuals differ in ability to perceive it (Lison et al. 1980; Hoffenberg 1983; Richer et al. 1989). There is precedent for the inability of some people with an otherwise normal sense of smell to fail to detect a particular odor, a genetic trait known as a specific anosmia (Guillot 1948; Amoore 1963). Furthermore, a specific anosmia for a sulfurous odor similar to the odor of the metabolites found in asparagus urine has been reported (Patterson and Lauder 1948). Recently, alleles of an olfactory receptor gene were shown to be associated with the perception of the odor or production of the asparagus odorant in urine (Eriksson et al. 2010). Subjects answered the question "Have you ever noticed a peculiar odor when you pee after eating asparagus?" From this question, it is not possible to tell whether the allele is related to the inability to produce the odorant (in an amount sufficient to detect) or the inability to smell it.

The production and the perception hypotheses are often treated as if they are mutually exclusive, although there is no reason why individual differences in both production and perception

Table 1 Proposed odorants found in asparagus urine

Compound	Reference
Methanethiol[a]	Nencki (1891)
Methanethiol	Allison and McWhirter (1956)
Methanethiol	Waring et al. (1987)
Methanethiol	Leitner (2001)
1-Propene-3-isothiocyante	Leitner (2001)
3-Methylthiophene	Leitner (2001)
Bis-(methythio)methane	Waring et al. (1987)
Carbon disulfide	Leitner (2001)
Carbon oxide sulfide	Leitner (2001)
Dimethyl disulfide	Waring et al. (1987)
Dimethyl disulfide	Leitner (2001)
Dimethyl sulfide	Leitner (2001)
Dimethyl sulfide	Waring et al. (1987)
Dimethyl sulfone	Stevens (2007)
Dimethyl sulfone	Waring et al. (1987)
Dimethyl sulfoxide	Waring et al. (1987)
Dimethyl trisulfide	Stevens (2007)
Dimethyl trisulfide	White (1975)
E-methylthio-1-propene	Leitner (2001)
Hydrogensulfide	Leitner (2001)
Methylpropylsulfide	Leitner (2001)
S-methyl-2-propenthioate	Leitner (2001)
S-methyl-2-propenethioate	Stevens (2007)
S-methyl-3-(methylthio)thiopropionate	White (1975)
S-methyl-thioacrylate	White (1975)
Tetrahydrothiophene	White (1975)
Methanesulfonic anhydride	Stevens (2007)
Butyrolactone	Stevens (2007)
1,4-bis(methythio)-butane	Stevens (2007)

[a]Also known as methyl mercaptan. 1,2-Dithiolane-4-carboxylic acid (asparagusic acid) is found in asparagus and may be the precursor to some of the sulfur metabolites listed above (Jansen 1948). The most common odorant detected in asparagus urine is methanethiol, listed at the top, followed by the other odorants in alphanumerical order.

cannot both be traits in the population. They might be due to the same cause. There is precedent for this hypothesis, as well: there are enzymes in human olfactory mucosa that alter molecules in ways that may change their odorant quality (Schilling 2006). Therefore, if a person lacks a key metabolic enzyme in the asparagus pathway, the inability to produce enough odorant to be detected in urine could also render the person unable to detect it (in any amount) by smell. In other words, the same enzyme could participate in both urine odorant production and in its detection. They could also coexist but be unrelated.

Because there is no known clinical problem associated with the inability to either excrete or detect the asparagus odor, the trait has received only scattered attention. The few population estimates of the ability to excrete the odorant in detectable quantities in urine (Table 2) and the ability to smell it (Table 3) vary widely. These discrepancies could be due to 1) poorly characterized or unreliable methods used to measure odorant production and odor perception, 2) genetic differences in trait frequencies among racial groups, 3) differential exposure to the odorant, or 4) a combination of any of these explanations. Because there are no standard methods of testing for odorous urine, earlier investigators sometimes asked subjects to distinguish between plain water and a dilute asparagus urine sample (Hoffenberg 1983). The drawback of this method is that subjects may be attending to urine odors rather than the asparagus feature. Other investigators asked subjects if they smelled an unusual odor from asparagus urine (Sugarman and Neelon 1985), which is prone to false-positive results because many plain urine samples might be considered to have an unusual or distinct odor. Another limitation of earlier work is that people were usually not tested for both their ability to produce and to perceive the odor. Finally, some studies overgeneralized the results of a few subjects to a larger population (Lison et al. 1980).

Given the contradictions in the literature, the purpose of our study was to develop a sensitive and unbiased psychophysical method to measure individual differences in the production of the odorants underlying the asparagus odor and in their perception. Subjects provided plain and asparagus urine for evaluation by themselves and by other subjects and in turn were asked to detect the asparagus odor from the urine of other people. To that end, we used a 2-alternative forced-choice technique in which subjects had to choose between the asparagus or plain urine. This procedure allowed us to determine whether an individual subject failed to produce the odorant or failed to perceive it, or both. To ensure that people who could not detect the asparagus odor were not generally insensitive to aromas, the threshold for phenyl ethyl alcohol was determined following methods used by the Monell-Jefferson Chemosensory Clinical Research Center (Cowart et al. 1997). We also obtained a DNA sample from each subject and genotyped them for the allele previously associated with the detection of the asparagus odor from urine. The purpose was to determine whether the genotype–phenotype association was for the ability to produce the underlying odorants and/or smell the odor.

Materials and methods

Overview of experimental procedure and timeline

Subjects came to the laboratory in the morning on 2 separate occasions at least 3 days apart and donated urine samples

Table 2 Summary of previous studies of odor production after asparagus consumption

Raters of odor[a]	*N* (F/M)[b]	Population	% Cannot produce[c]	Reference
Not given	103 (50F/53M)	French	0	Richer et al. (1989)
Study authors	19 (12F/7M)	American	21	Sugarman and Neelon (1985)
3 Judges	800 (238F/562M)	British	57	Mitchell et al. (1987)
Not given	115 (not given)	British	60	Allison and McWhirter (1956)
Gas chromatography	3 (3F)	American	67	Gearhart et al. (1977)

[a]"Raters of odor" refers to the people or instrumentation classifying the presence or absence of asparagus odor from urine.
[b]*N*, sample size; M, male; F, female.
[c]"% Cannot produce" indicates cannot produce the characteristic compounds associated with the odor from asparagus urine. Psychophysical methods used to detect the asparagus odor are either not given (Allison and McWhirter 1956; Richer et al. 1989) or briefly described. Typically, subjects were allowed to sniff the urine and asked to decide if it had an "unusual" (Sugarman and Neelon 1985) or "characteristic" odor (Mitchell et al. 1987).

Table 3 Summary of previous studies of asparagus urine odor perception

Raters of odor	Method used[a]	Test	*N* (F/M)	Population	% Cannot smell	Reference
Subjects	Other's Urine	Dilute urine versus water	328 (not given)	Israeli	0	Lison et al. (1980)
Subjects	Other's Urine	Dilute urine versus water	98 (52F/46M)	Chinese	2	Hoffenberg (1983)
Subjects	Other's Urine & Own Urine	Undiluted urine	15 (not given)	American	33	Sugarman and Neelon (1985)
Subjects	Other's Urine	Dilute urine versus water	21 (not given)	American	50[b]	Lison et al. (1980)

See Table 2 for abbreviations. All subjects in Tables 2 and 3 were adults except for one study of children (Hoffenberg 1983).
[a]Two methods are used; the subject either smelled the urine of someone else (Other's Urine) or smelled their own urine (Own urine). Psychophysiological methodologies were of 2 types: the subjects were either forced to choose between dilute urine and water (Lison et al. 1980) or asked to smell urine and report an "unusual" (Sugarman and Neelon 1985) or "special" odor (Hoffenberg 1983).
[b]Subjects may have been selected to have equal numbers of people who could and could not smell the asparagus odor.

before and after eating asparagus or bread. Subjects then returned to the laboratory on subsequent days, after urines for all participants were collected, and evaluated the urine samples using the forced choice procedure described below. On the last day, subjects were tested for olfactory sensitivity. Subjects in the first experiment (hereafter Experiment 1) were not tested further, but subjects in Experiment 2 performed an additional task, also described below. Experiments 1 and 2 were conducted about 4 months apart, and no subject from Experiment 1 participated in Experiment 2.

Subjects

Adult subjects were recruited by local newspaper advertisement, by flyers placed near the Monell Chemical Senses Center, and by word of mouth and were screened either by telephone or by personal interview to determine whether they were eligible to participate. Pregnant women and people younger than 18 years of age or older than 65 years of age were excluded from participation. Subjects completed a brief questionnaire with demographic questions, including whether they were current smokers. The experimental protocol was approved by the University of Pennsylvania's Institutional Review Board for Research with Human Subjects, and all participants in the study provided written informed consent and were paid for their participation.

Thirty-eight adult men and women participated in this study. Some subjects were unable to complete some parts of the testing. For instance, some people could not complete the smelling phase because of unanticipated aversions to urine or lack of availability to complete testing. Table 4 contains age, race, and sex data for all subjects.

Consumption of asparagus and urine collection

Urine was collected on 2 separate days. Subjects were asked to come to the laboratory at 10 AM and were allowed to eat breakfast beforehand with the proviso that they ate the same breakfast at the same time on both urine collection days and that they did not eat asparagus in the prior 24 h. On 1 day, upon arrival in the laboratory, subjects provided a urine sample collected in a plastic beaker (the before-asparagus sample). Immediately upon voiding, the urine sample was transferred to a glass jar and frozen at –20 °C. At approximately 10:30 AM, the subject then ate roasted asparagus and drank a 16-ounce bottle of water. The raw asparagus (125 g) was prepared by combining it with 1 teaspoon of extra virgin olive oil (Colavita brand) and 0.8 g kosher salt and broiling it for 8 min. Two hours after asparagus

Table 4 Individual subjects with phenotype and genotype data

Subject ID	Experiment	Sex	Race	Age	Odorant perception	Odorant production	*rs4481887*
117	1	F	AA	26	0.69	0.63*	GG
101	1	M	AA	31	0.75	0.89	GG
315	2	F	AA	20	0.79	0.82	GG
118	1	M	AA	52	0.90	0.82	GG
106	2	F	AA	34	0.96	0.91	GG
114	1	M	AA	52	0.98	0.99	GG
318	2	M	AS	41	0.79	0.88	GG
109	1	M	AS	55	0.86	0.94	AG
111	1	F	AS	36	0.95	0.92	GG
115	1	M	AS	34	0.99	0.99	GG
313	2	F	CA	42	0.51*	0.74	GG
306	2	M	CA	57	0.69	0.97	GG
312	2	M	CA	49	0.71	0.88	GG
308	2	M	CA	24	0.80	0.76	AG
113	1	M	CA	27	0.81	0.82	AG
310	2	F	CA	29	0.82	0.87	AG
304	2	F	CA	27	0.83	0.87	GG
108	1	M	CA	26	0.85	0.79	AG
303	2	M	CA	34	0.87	ND	AG
116	1	M	CA	40	0.88	0.99	GG
104	1	F	CA	24	0.89	0.93	AG
202	1	F	CA	43	0.89	0.93	AG
103	1	F	CA	22	0.91	0.97	GG
201	1	F	CA	49	0.93	0.57*	AG
102	1	F	CA	23	0.96	0.89	AA
105	1	F	CA	25	0.96	0.90	AG
311	2	M	CA	31	0.96	0.90	AG
302	2	F	CA	23	0.97	0.60*	AG
309	2	F	CA	27	0.99	0.81	AG
305	2	F	CA	37	ND	0.79	AG
314	2	F	CA	24	ND	0.79	GG
107	1	M	CA	22	ND	0.87	AG
317	2	F	CA	25	ND	0.90	GG
307	2	M	OT	28	ND	0.76	AG
316	2	F	CA	29	0.94	0.91	ND
301	2	F	AA	43	0.58*	0.80	ND
112	1	F	CA	24	ND	0.91	ND
110	1	M	CA	25	ND	0.96	ND

Subject IDs are listed by anonymous identifier. See text for a description of the differences between Experiments 1 and 2. M, male; F, female. Subjects self-identified their race, CA, Caucasian; AA, African-American; AS, Asian; OT, Other. Odorant perception, the proportion of trials in which subjects could correctly identify the urine collected after asparagus consumption. Odorant production, the proportion of trials subjects could distinguish, for that individual, the "before-" versus "after-" asparagus urine. *rs4481887* is the unique identifier of the genetic variant typed near the olfactory receptor *OR2M7*. GG, homozygous for the major allele; AG, heterozygous; AA, homozygous for the minor allele. Some data are missing because subjects declined to participate in some parts of the experiment or provide certain data. Values that do not depart from those expected by chance are double underlined with an asterisk (*) and indicate that subjects cannot produce the asparagus odorant in sufficient quantities to be detected or cannot detect it in the urine of others. Values that reflect greater than chance performance but that are still worse than the majority of the subjects are single underlined. Subjects are grouped by race and ordered by odorant perception from least to most sensitive. Subjects for whom a genotype could not be obtained are listed at the bottom. See text for other details.

ingestion, the subjects provided a second urine sample (after-asparagus sample).

On the other day, subjects arrived at the laboratory at the same time (10 AM), provided a urine sample in the same manner and then ate bread (a 72 g Italian bread roll with the same amounts of added salt and oil) and drank 16 ounces of water. Two hours after the bread was eaten, another urine sample was collected. The bread day and the asparagus day were in counterbalanced order.

Urine sensory testing

The goal of testing was to determine whether subjects could reliably choose, when presented with 2 samples, the one collected after eating asparagus. The testing procedure was broken into multiple sessions, and in each session, the subject smelled the urine from a single subject (including one session in which they smelled their own urine). Each session was short, 15 min in length or less, and was scheduled at the convenience of the subjects but normally took place from 9 AM to 5 PM during the workweek. Subjects were allowed to perform 2 sessions in 1 day, but each session was separated by at least 1 h to prevent olfactory fatigue. Although all subjects agreed to smell their own urine and the urine of other people upon enrollment into the study, several people were actually unavailable to do so by the time all urines were collected. These subjects were excused from this portion of the study.

For each session, the urine samples were defrosted at 6–7 °C overnight, allowed to come to room temperature (20 ± 1 °C) during the 90 min preceding testing, gently stirred, and 6 mL of each urine sample was transferred into a 2-ounce glass bottle. No attempt was made to control the volume of urine produced by a subject or to dilute or concentrate the urine samples. The bottom and sides of the glass bottle were covered with aluminum foil to inhibit degradation of the sample by light, and the top was covered with a layer of gauze to prevent the subjects from seeing the urine. The gauze had a loose weave so that volatile molecules could freely disperse into the airspace sniffed by the subjects. Subjects and experimenters both wore white cotton gloves to reduce smells from their skin, for example, lotions or soaps.

Subjects were first asked to sniff the asparagus urine from a particular subject and were told that this was the ''urine produced after eating asparagus.'' Next the subject was given 2 bottles of urine from that subject, prepared as described above, and instructed to sniff each bottle in turn. One bottle containing urine was collected after asparagus ingestion and a second bottle of urine was collected after bread ingestion, and they were asked to select the jar that contained the asparagus odor, and if they were unsure, they were instructed to guess. Likewise, subjects were offered a different type of choice between 2 bottles, one of which contained urine collected before and one of which contained urine collected after asparagus ingestion, again indicating which one had the asparagus odor. The subject selected 1 of the 2 bottles, and the choice was recorded by the investigator. For each session, subjects were offered each type of choice 3 times for a total of 6 choices. Preliminary data analyses indicated that there were no significant differences between the subjects' abilities to detect the asparagus odor regardless of the type of non-asparagus control urine (for Study 1, $t(11) = -0.43$, not significant [NS]). Therefore, data from both types of choices were combined, and each subject received a score that reflected the number correct out of 6 choices. This 2-alternative forced-choice testing procedure was the same for subjects in Experiment 1 and Experiment 2.

Tests of general olfactory function

Because olfactory ability can be influenced by a variety of factors, it was important to ensure that each subject had a normal sense of smell. Therefore, we measured olfactory detection thresholds to phenyl ethyl alcohol to provide an evaluation of general olfactory function using the same odorant and methods used in the clinical assessment conducted at the Monell-Jefferson Chemosensory Clinical Research Center (Cowart et al. 1997). This odor was selected for clinical testing because no specific anosmia to it had been described and because it does not elicit a trigeminal (irritation) response (and thus threshold sensitivity was believed to reflect only olfactory ability). To that end, phenyl ethyl alcohol (rose; Sigma P-6134) was diluted in 20 half-log dilution steps starting from 100% pure odorant. The blank and diluent were glycerol (Sigma G9012), and samples (20 mL) were presented to subjects in 300-mL polypropylene squeeze bottles. Thresholds were determined by a forced-choice staircase procedure. For each trial, the bottle with no odor and the bottle with the odor were offered to the subjects. They were asked to indicate the bottle they thought had the odor. Following one wrong response an increased concentration was offered on the next trial, whereas 2 correct responses resulted in a decrease of concentration. A ''reversal'' was when the concentration sequence changed from decreasing to increasing or vice versa, and the testing was ended after 5 reversals. Thresholds were calculated as the average of the dilution step values of the last 4 reversals. The threshold of each subject was compared with those obtained from clinically normal control subjects, and those 3 standard deviations from the mean (in the less sensitive direction) were considered to have impaired olfactory function and were removed from the analysis. In fact, no subjects met this criterion, and none were removed.

Detection of basil odor added to urine

Because smelling the urine of others is an unfamiliar task, after Experiment 1, we decided to add an additional task to determine whether subjects were able to follow instructions and detect an unrelated but unusual odor added to urine. Therefore, subjects in Experiment 2 completed an extra sensory test in which they were asked to smell urine samples with and without an added odor (basil) and choose the sample with the added odor. With one exception explained below, the sensory testing was conducted in the same manner as the asparagus testing except, instead of choosing between asparagus and plain urine, they chose between urine spiked with a basil odor (50 μL of liquid basil extract; McCormick brand) and plain urine. The basil odor was chosen for practical reasons, it was readily available, and it had a distinctive odor to the investigators and was a mixture of many volatile compounds. As such, most people would be able to smell at least some of its odorants. The exception mentioned above was that whereas in the asparagus sensory testing, subjects smelled the urine of many subjects (in separate sessions), for this sensory test, only one subject's urine was tested. This urine was from a subject who had not eaten asparagus in the previous 24 h.

DNA collection, marker selection, and genotyping

Cells from the cheek were obtained from each subject, and genomic DNA was extracted following the directions of the manufacturer (Epicenter). The marker *rs4481887* was selected to genotype because it was previously associated with the ability to perceive the unusual odor of asparagus urine (Eriksson et al. 2010). This variant site is at the extreme telomere of the long arm of chromosome 1 and is in the middle of a 1.6-Mb region, which contains a cluster of 50 olfactory genes. Variant sites in this region are in high linkage disequilibrium. The polymorphic marker *rs4481887* is between 2 olfactory receptor genes, *OR2M7* and *OR14C36*, but is slightly closer to *OR2M7* (GRCh37; Figure 1). Alleles of this marker were genotyped using allele-specific probes and primers purchased from Applied Biosystems (Catalogue # C__26719686_10). Assays were run in duplicate using an OneStep from Applied Biosystems, and in no cases were discrepancies in genotype noted between duplicates. No attempt was made to more precisely map the trait because the strength of the linkage disequilibrium and the small sample size made it unlikely that fine mapping would be successful.

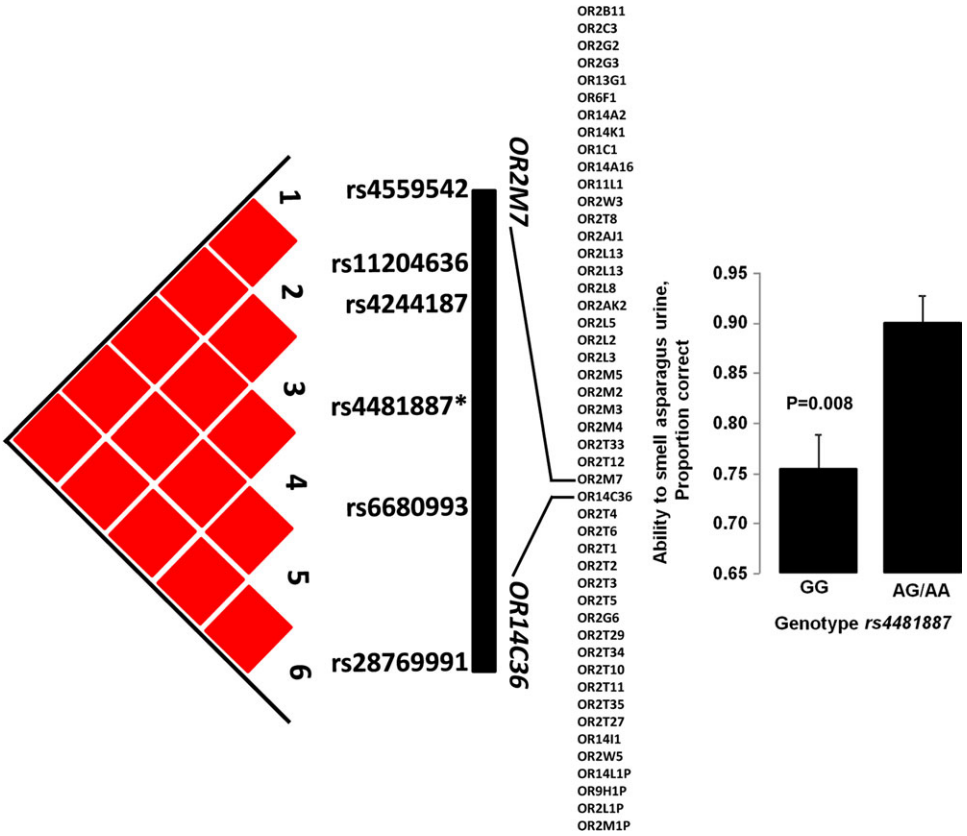

Figure 1 Genetic association between alleles of *rs4481887* and the ability to smell the odor of asparagus urine. The list of olfactory receptor genes comprises the cluster in a 1.6-Mb region of chromosome 1q44. The region between the olfactory receptor gene *OR2M7* and *OR14C36* is shown in detail, with indices of linkage disequilibrium among markers in square boxes ($D' > 0.92$). The variant genotyped is indicated by an asterisk (*). This figure appears in color in the online version of *Chemical Senses*.

Data analysis

The main outcome variable in the study was the number of correct responses per urine sample (failure to produce the asparagus odorant) or per subject (failure to smell the asparagus odor). To determine whether a person was unable to produce asparagus odorants in urine, we asked whether the proportion of correct answers about each sample was different than chance (0.5 = chance) using a repeated-measures analysis of variance (ANOVA) and Tukey post hoc tests. To determine whether a person was unable to smell the asparagus odor in urine, we determined whether the proportion of correct answers by a subject was different than expected by chance (0.5 = chance) generating a t statistic with degrees of freedom equal to number of sessions rating the urine minus 1. A P value of <0.05 was considered statistically significant.

For peripheral olfactory sensitivity, the threshold of each person was calculated as described above and compared with a reference clinical population. To understand whether individual differences within the normal range of peripheral olfactory acuity were related to anosmia for the asparagus odor, an index of performance on asparagus perception (proportion of correct choices) was correlated with the olfactory threshold for phenyl ethyl alcohol and the resulting r value tested to see if it differed from zero (Edwards 1973). To determine whether the failure to correctly identify the asparagus odor in urine was due to an inability to follow the instructions, a correlation coefficient was calculated between the subject's performance in the asparagus and basil sensory tests and the r value tested for significance as described above (Experiment 2 only). In addition, the subjects from Experiments 1 and 2 were pooled, and the frequencies of odor production and perception were calculated, [(number of subjects who failed to produce the odor/total number of subjects in Experiments 1 and 2) × 100].

For the genetic association analysis, the abilities to produce and detect the odor of asparagus in urine were treated as quantitative traits, with the proportions of correct choices as the dependent variables. For perception, proportion correct refers to the number of times the subject correctly chose the asparagus from plain urine out of the total number of trials. For odorant production, the proportion correct refers is the number of times the asparagus odorant could be detected by others. Because the protocol in Experiments 1 and 2 did not vary for the collection of the dependent variables, the data were combined for the genotype–phenotype analyses. The original association was reported for people

of European ancestry (Eriksson et al. 2010), so only Caucasians were included in this analysis. Subjects were grouped by *rs4481887* genotype and compared for the proportion correct with a *t*-test. Heterozygotes (AG; $N = 14$) and homozygotes for the minor allele (AA; $N = 1$) were collapsed into a single group and compared with those with 2 copies of the major allele (GG; $N = 9$). Eta squared (η^2) is a measure of effect size and was calculated here to establish the percentage of variance accounted for by genotype.

Results

Odor production

In Experiment 1, one of the subjects did not excrete the asparagus odorant at a concentration high enough to be detected. In other words, subjects could detect the asparagus odor at greater than chance frequency for all but one subject's urine, *t* values ranging from $t(15) = 1.81$, $P = 0.08$ (the person who failed to produce the odor) to $t(15) = 47.13$, $P < 0.01$. In Experiment 2, the results were similar: 2 subjects failed to produce the asparagus odor in sufficient amounts to be detected by other subjects, with *t* values ranging from $t(14) = 1.07$, $P = 0.3$ to $t(14) = 21.4$, $P < 0.001$ (Table 4). For the remainder of the subjects, the smell of asparagus metabolites could be reliably detected, all *P* values less than 0.05.

The asparagus odor was more obvious in some samples than others. We performed a repeated-measures ANOVA across subjects, with the dependent variable as the proportion of correct identifications of the asparagus urine for each session. We found differences across subjects in the average proportion of urines correctly identified (Experiment 1: $F(17,255) = 4.02$, $P < 0.001$, Experiment 2: $F(18,252) = 4.92$, $P < 0.001$). Tukey tests showed that one subject in Experiment 1 and 2 subjects in Experiment 2 had asparagus urines that were not identified as well as others (Table 4).

Odor perception

In Experiment 1, all subjects were able to smell the asparagus odor in human urine and correctly chose the urine sample with the asparagus odor at greater than chance frequencies (*t* values ranged from a low of $t(17) = 2.7$, $P < 0.02$ to a high of $t(17) = 53.00$, $P < 0.001$). In Experiment 2, 2 subjects did not distinguish the asparagus urine from other urines at better than chance levels (*t* values ranged from a low of $t(18) = 0.22$, NS to $t(18) = 56$, $P < 0.001$; Table 4). Whereas only 2 people were unable to detect the asparagus odor, there was a range in the ability of subjects to detect the asparagus urine. ANOVA followed by Tukey tests showed that 2 subjects from Experiment 1 were significantly less accurate than others, though they performed better than chance ($F(15,255) = 3.98$, $P < 0.0001$). Similar results were obtained in Experiment 2 ($F(14,252) = 14.32$, $P < 0.0001$); 4 subjects (including the 2 who were anosmic to the asparagus metabolite, mentioned above) were significantly less accurate than the others (Table 4). There was no sex difference in either the ability to smell the odorants or produce them nor were there any reliable relationships between age and these traits (all *P* values > 0.05).

To determine whether people who could not smell the asparagus urine had an otherwise normal sense of smell, a threshold for phenyl ethyl alcohol (rose) was determined. All thresholds were within 3 standard deviations of clinically normal results, and no subject was excluded from Experiment 1 or Experiment 2. Further analyses of these data suggested that the threshold for the phenyl ethyl alcohol was unrelated to whether a subject could detect the asparagus odor in urine (Experiment 1, $r(16) = 0.18$, NS; in Experiment 2, $r(15) = -0.06$, NS).

Detection of the basil odor added to urine

All subjects in Experiment 2 distinguished the basil-spiked urine from plain urine almost perfectly. The average proportion correct was 0.96 ± 0.07, and the range was 0.83–1.00 (a proportion of 1.00 means that a subject was picked the correct sample 6 out of 6 times). There was no relationship between performance on the basil and on the asparagus task ($r(15) = -0.05$, NS); therefore, it is unlikely that the subjects who repeatedly failed to choose the asparagus urine over the plain urine did so because they did not understand the instructions.

Co-occurrence of production and perception

Combining the data from Experiments 1 and 2, 3 people out of the 37 who provided urine were unable to produce the asparagus odorant (8.1%). Likewise, 2 people out of 31 who participated in the sensory tests failed to detect the odor (6.4%). One person showed evidence of both the failure to produce the odorant and to perceive the after-asparagus odor (3.1%). The correlation between perception and production was not different than zero ($r = 0.24$, $P = 0.197$).

Genetic association

There were racial differences in *rs4481887* allele frequency with Caucasian subjects having a minor allele frequency of 0.35, whereas there was no observed genetic variation in subjects of African descent (all genotypes were GG). Figure 1 shows the ability of Caucasian subjects grouped by *rs4481887* genotype to detect the asparagus odorant (measured by the proportion of trials they correctly identified the asparagus from plain urine). Individual data for these subjects as well as those from other racial groups are presented in Table 4. Genotypes near the *OR2M7* gene were related to the ability to smell the asparagus odor ($t(17) = 8.93$, $P = 0.008$) but not to the ability to produce it ($t(1,20) = 2.43$, $P = 0.13$). One-third of the variance among Caucasians is explained by alleles at this location. The A allele was associated with greater ability to detect the asparagus odorant, which is the same allele which was associated with this ability in a previous study (Eriksson et al. 2010).

Discussion

When humans eat asparagus, some people report a distinct odor afterward from their urine. About 8% of the subjects studied herein did not produce this characteristic asparagus odor in sufficient concentration to be detected by the methods used here. However, the recognition of the asparagus odor in urine is not an all-or-nothing phenomenon; some people produce an asparagus odor that is easy to detect, and the presumption is that some people produce more odorant. However, because the odor-causing molecules have not been unequivocally identified (see Table 1), it is not possible to measure its concentration in urine; it is reasonable to assume that odorant production varies from individual to individual, and people with urine that does not have a detectable odor may produce it, albeit at a low concentration. Part of the difference in odorant production could also be due to the production by some people of less volatile variants.

About 6% of subjects are unable to detect asparagus odor, and we ruled out generalized smell loss in these subjects by assessing their response to a second odorant. Therefore, the most likely explanation is that these individual differences in odor detection are a specific anosmia. Specific anosmias are common for biologically important odors, such as volatile steroid hormones, musk, and sweat (Guillot 1948; Amoore 1963; Amoore et al. 1975; Amoore and Forrester 1976; Baydar et al. 1992, 1993; Gilbert and Kemp 1996), and the smell of human urine in different nutritional states, for example, after asparagus consumption. The presumption is that one or more olfactory receptors respond to the asparagus odor but that these receptors are less functional in some people. To the best of our knowledge, there have been no family or twin studies of this anosmia, but differences in olfactory ability are due to heritable variation in olfactory receptors (Keller et al. 2007; Menashe et al. 2007), so this genetic explanation fits the available data (Eriksson et al. 2010). Although specific anosmias are often thought of as being all-or-none traits, thresholds are on a continuum and that is likely to be the case here. Some people are much less sensitive than others, but they may be able to smell the odorant if it were at higher concentrations than are usually found in human urine.

Odor sensitivity can change with repeated exposure to the odorant (Wysocki et al. 1989; Dalton et al. 2002), so someone who cannot produce the asparagus odor might be less able to smell it because they have less experience with the odor from their own urine. We cannot rule out the hypothesis that exposure to the asparagus odorant makes people more sensitive, and one limitation of this study was the lack of information about habitual asparagus intake. It is possible that genotype by experience effects might be important and that people with sensitive genotypes might become even more sensitive if they eat asparagus frequently and often produce (and smell) the odor. Future research should include the frequency of asparagus consumption to help determine the effect of experience on its perception.

We chose a 2-h window after asparagus consumption to collect urine. This time point was chosen based on bench testing, which suggested that the asparagus odor appeared at maximal intensity within this window, although it is probable that there are also individual differences in the appearance rate of the odor as well as its peak intensity. The urine odorant produced after asparagus ingestion may be a metabolic product or it may be a molecule found in cooked asparagus that is eliminated unchanged (Ulrich et al. 2001). A better understanding of the time course of odor production would provide a clue about its origins (Gautier 1923).

The rates of specific anosmia for the asparagus odor in this study were generally lower than those reported by other investigators (Table 3). This difference may be due, in part, to our use of a 2-alternative forced choice procedure, which is less prone to certain types of bias. For instance, subjects asked whether they smelled an unusual odor in urine might be inclined to answer ''yes'' regardless of whether they could detect the specific odor. Using the methods herein (by forcing the subjects to choose between 2 samples), we can be more confident that, if they gave a correct answer on 6 occasions, they smelled the target odor. Likewise, fewer people failed to produce the odor in this study compared with previous reports, and this may be due, in part, to the use of the 2-alternative forced choice method, which allows subjects to directly compare asparagus and plain urine. This is a more sensitive test compared with other methods and resulted in lower, but perhaps more accurate, rates of failure to produce the characteristic asparagus odor at high enough concentrations to be perceived in these test conditions.

The reduced ability to smell the asparagus metabolites in urine appears to be related to a single nucleotide polymorphism near the olfactory receptor gene *OR2M7*. This genotype–phenotype relationship is similar to other alleles in olfactory receptors that reduce the ability to smell androstenone (Keller et al. 2007) and isovaleric acid (Menashe et al. 2007). The polymorphism lies within a large cluster of olfactory receptors on chromosome 1q44 that contains many alleles, most of which are in high linkage disequilibrium with alleles in and near other olfactory receptor genes. *OR2M7* itself responds to the odorants geraniol and cintrone, which have a rose and citrus quality (Saito et al. 2009). The compounds that cause the asparagus odor have not been unequivocally identified; it is not possible to directly test whether the *OR2M7* olfactory receptor itself or a neighboring one responds to asparagus odorant.

The genetics of odor production for some particular chemicals is well understood. For instance, the fish odor associated with trimethylaminuria is related to alleles of the *FM03* gene (Dolphin et al. 1997). People also differ in their propensity to produce axillary odor in part due to alleles of the *ABCC11* gene (Martin et al. 2010). In this study, alleles of an olfactory receptor were not significantly related to the

ability to produce the asparagus urine odor (although small effects might not have been detected). The major determinant of individual differences in asparagus odor production in urine remains unknown. In conclusion, this study confirmed that people with a particular allele within an olfactory gene cluster is related to the ability to smell the odor. We also report that the production of the asparagus metabolites was not tightly related to the ability to smell them.

Funding

Monell Chemical Senses Center institutional resources.

Acknowledgements

The authors thank Drs George Preti, Johannes Reisert, Charles Wysocki, Antti Knaapila, and Gary K. Beauchamp for comments on an earlier draft of the manuscript. Jessica Jane Tordoff provided assistance with French language translation. The editorial assistance of Patricia J. Watson and the suggestions made by Doron Lancet, Joanna Mountain, Nicholas Eriksson, and 2 anonymous reviewers are gratefully acknowledged.

References

Allison AC, McWhirter KG. 1956. Two unifactorial characters for which man is polymorphic. Nature. 178:748–749.

Amoore JE. 1963. Stereochemical theory of olfaction. Nature. 199:912–913.

Amoore JE, Forrester LJ. 1976. Specific anosmia to trimethylamine: the fishy primary odor. J Chem Ecol. 2:49–56.

Amoore JE, Forrester LJ, Buttery RG. 1975. Specific anosmia to 1-pyrroline: the spermous primary odor. J Chem Ecol. 1:299–310.

Baydar A, Petrzilka M, Schott M-P. 1992. Perception of characteristic axillary odors. Perfum Flavor. 17:2–9.

Baydar A, Petrzilka M, Schott M-P. 1993. Olfactory thresholds for androstenone and Galaxolide: senstivity, insensitivity and specific anosmia. Chem Senses. 18:661–668.

Cowart BJ, Young IM, Feldman RS, Lowry LD. 1997. Clinical disorders of smell and taste. Occup Med. 12:465–483.

Dalton P, Doolittle N, Breslin PA. 2002. Gender-specific induction of enhanced sensitivity to odors. Nat Neurosci. 5:199–200.

Dolphin CT, Janmohamed A, Smith RL, Shephard EA, Phillips IR. 1997. Missense mutation in flavin-containing mono-oxygenase 3 gene, FMO3, underlies fish-odour syndrome. Nat Genet. 17:491–494.

Edwards AL. 1973. Statistical methods. New York: Holt, Rinehart and Winston.

Eriksson N, Macpherson JM, Tung J, Hon L, Naughton B, Saxonov S, Avey L, Wojcicki A, Pe'er I, Mountain J. 2010. Web-based, participant-driven studies yield novel genetic associations for common traits. PLoS Genet. 6(6):e1000993.

Franklin B, Japikse C. 2003. Fart proudly: writings of Benjamin Franklin you never read in school. Berkeley (CA): Frog Enthea Press.

Gautier C. 1923. Rapidite d'elimination urinaire du methylmercaptan apres ingestion d'asperges. C R Seances Soc Biol Fil. 89:239.

Gearhart HL, Pierce SK, Payne-Bose D. 1977. Volatile organic components in human urine after ingestion of asparagus. Clin Chem. 23:1941.

Gilbert AN, Kemp SE. 1996. Odor perception phenotypes: multiple, specific hyperosmias to musks. Chem Senses. 21:411–416.

Guillot M. 1948. Physiologie Des Sensations. C R Congr Annu Med Electro-Radiol Lang Fr. 226:1307–1309.

Hoffenberg L. 1983. A note on polymorphism: the ability to smell urinary metabolites of asparagus. Diastema. 11:37–38.

Humbert JA, Hammond KB, Hathaway WE. 1970. Trimethylaminuria: the fish-odour syndrome. Lancet. 2:770–771.

Jansen EF. 1948. The isolation and identification of 2,2′-dithiolisobutyric acid from asparagus. J Biol Chem. 176:657–664.

Keller A, Zhuang H, Chi Q, Vosshall LB, Matsunami H. 2007. Genetic variation in a human odorant receptor alters odour perception. Nature. 449:468–472.

Leitner E. 2001. Identification of odorous compounds in urine after the consumption of asparagus. Recent Res Dev Agric Food Chem. 5:161–166.

Lison M, Blondheim SH, Melmed RN. 1980. A polymorphism of the ability to smell urinary metabolites of asparagus. Br Med J. 281:1676–1678.

Martin A, Saathoff M, Kuhn F, Max H, Terstegen L, Natsch A. 2010. A functional ABCC11 allele is essential in the biochemical formation of human axillary odor. J Invest Dermatol. 130:529–540.

Menashe I, Abaffy T, Hasin Y, Goshen S, Yahalom V, Luetje CW, Lancet D. 2007. Genetic elucidation of human hyperosmia to isovaleric acid. PLoS Biol. 5:e284.

Mitchell SC. 2001. Food idiosyncrasies: beetroot and asparagus. Drug Metab Dispos. 29:539–543.

Mitchell SC, Waring RH, Land D, Thorpe WV. 1987. Odorous urine following asparagus ingestion in man. Experientia. 43:382–383.

Nencki M. 1891. Ueber das vorkommen von methylmercaptan in menschlichen harn nach spargelgenuss. Arch Exp Path Pharmak. 28:206–209.

Patterson PM, Lauder BA. 1948. The incidence and probable inheritance of smell blindness to normal butyl mercaptan. J Hered. 39:295–297.

Proust M. 1929. Du cote de chez Swann. A la recherche du temps perdu, I. Paris: Gallimard, Editions de la Nouvelle Revue Francaise. p. 67.

Richer C, Decker N, Belin J, Imbs JL, Montastruc JL, Giudicelli JF. 1989. Odorous urine in man after asparagus. Br J Clin Pharmacol. 27:640–641.

Saito H, Chi Q, Zhuang H, Matsunami H, Mainland JD. 2009. Odor coding by a Mammalian receptor repertoire. Sci Signal. 2:ra9.

Schilling B. 2006. Method to identify or evaluate compounds useful in the field of fragrances and aromas. UK. p. 29.

Stevens P. 2007. Detection of sulfur-containing metabolites of asparagus in human urine by SBSE-GCxGC-TOFMS. LCGC North America, Suppl., 16.

Sugarman J, Neelon FA. 1985. You're in for a treat: asparagus. N C Med J. 46:332–334.

Ulrich D, Hoberg E, Bittner T, Engewald W, Meilchen K. 2001. Contribution of volatile compounds to the flavor of cooked asparagus. Eur Food Res Technol. 213:200–204.

Waring RH, Mitchell SC, Fenwick GR. 1987. The chemical nature of the urinary odour produced by man after asparagus ingestion. Xenobiotica. 17:1363–1371.

White RH. 1975. Occurrence of S-methyl thioesters in urines of humans after they have eaten asparagus. Science. 189:810–811.

Wysocki CJ, Dorries KM, Beauchamp GK. 1989. Ability to perceive androstenone can be acquired by ostensibly anosmic people. Proc Natl Acad Sci U S A. 86:7976–7978.