Sniffing out significant “Pee values”: genome wide association study of asparagus anosmia

Sarah C Markt,1 Elizabeth Nuttall,1 Constance Turman,4 Jennifer Sinnott,1,5 Eric B Rimm,1,2,6 Ethan Ecsedy,2 Robert H Unger,1 Katja Fall,1,8,9 Stephen Finn,10 Majken K Jensen,2,6 Jennifer R Rider,1,11 Peter Kraft,1,3,4 Lorelei A Mucci1,6,9

ABSTRACT

OBJECTIVE To determine the inherited factors associated with the ability to smell asparagus metabolites in urine.

DESIGN Genome wide association study.

SETTING Nurses’ Health Study and Health Professionals Follow-up Study cohorts.

PARTICIPANTS 6909 men and women of European-American descent with available genetic data from genome wide association studies.

MAIN OUTCOME MEASURE Participants were characterised as asparagus smellers if they strongly agreed with the prompt “after eating asparagus, you notice a strong characteristic odor in your urine,” and anosmic if otherwise. We calculated per-allele estimates of asparagus anosmia for about nine million single nucleotide polymorphisms using logistic regression. P values ≤10⁻⁵ were considered as genome wide significant.

RESULTS 58.0% of men (n=1449/2500) and 61.5% of women (n=2712/4409) had anosmia. 871 single nucleotide polymorphisms reached genome wide significance for asparagus anosmia, all in a region on chromosome 1 (1q44: 248139851-248595299) containing multiple genes in the olfactory receptor 2 (OR2) family. Conditional analyses revealed three independent markers associated with asparagus anosmia: rs13373863, rs71538191, and rs6689553.

CONCLUSION A large proportion of people have asparagus anosmia. Genetic variation near multiple olfactory receptor genes is associated with the ability of an individual to smell the metabolites of asparagus in urine. Future replication studies are necessary before considering targeted therapies to help anosmic people discover what they are missing.

WHAT IS ALREADY KNOWN ON THIS TOPIC

Although asparagus is considered a delicacy, it tends to endow human urine with distinctive odor.

The ability to smell the metabolites of asparagus consumption varies among people and across populations.

WHAT THIS STUDY ADDS

This study provides important knowledge of the inheritance of asparagus anosmia, and genetic variation was identified near multiple olfactory receptor genes.

Introduction

In 1781 Benjamin Franklin remarked, “a few stems of asparagus eaten, shall give our urine a disagreeable odour.”3-5 The consequence of asparagus consumption has been a topic of both public and private discussion, with Proust’s observation of asparagus spears perhaps the most poetic, “they played . . . at transforming my humble chamber into a bower of aromatic perfume.”6 For those who can detect the distinctive sulfurous odor it must seem, as the French botanist and chemist Louis Lémery wrote in 1702, “They [asparagus spears] cause a filthy and disagreeable smell in the urine, as everybody knows.”7

But not everybody does seem to know, as a subset of the population is unable to smell the methanethiol and S-methyl thioesters metabolites produced by asparagus consumption. It was uncertain whether this inability to detect the metabolites was related to a failure to produce the metabolites or to a specific anosmia. Foundational research found that the prevalence differs between people and across populations.6-8 Studies have shown that people who cannot smell the odor in their own urine are also unable to smell it in the urine of known producers,7 lending credence to the anosmia hypothesis. Regardless of whether the inability to detect the odor is a problem of perception or production, the phenotypic distribution suggests a potential genetic component.6,7

Few scientists have sought to examine the inherited factors associated with asparagus anosmia. In 2010 the results of a genome wide association study in 4727 participants was reported.10 The study found that a single nucleotide polymorphism—an individual genetic variation in DNA—rs4481887 located near olfactory receptor 2M7 (OR2M7) was statistically significantly associated with participant reported anosmia to asparagus.

Given the complexity of olfactory receptors it is likely that additional genetic variations contribute to the differences in humans’ ability to detect the odor of asparagus metabolites. We therefore carried out a genome wide association study of asparagus anosmia among two large and well characterized US based cohorts: the Nurses’ Health Study and the Health Professionals Follow-Up Study.

Methods

This study was conceived during a scientific meeting attended by several of the coauthors in bucolic Sweden, where it became apparent that some of us were unable to detect any unusual odor in our urine after consuming new spring asparagus. We subsequently sought
epidemiological studies to further examine this phenomenon, and found the Nurses’ Health Study and Health Professionals Follow-up Study (see supplementary file for details). The participants included in the current study included men and women of European descent with available genetic data from genome wide association studies from nested case-control studies. All participants gave informed consent, including consent for genetic analyses.

**Definition of asparagus anosmia**
The main outcome in our study was asparagus anosmia, which was collected in both the Nurses’ Health Study and the Health Professionals Follow-up Study as part of a broader supplemental questionnaire sent to participants in 2010. They were asked to respond to the prompt: “After eating asparagus, you notice a strong characteristic odor in your urine.” For the primary analysis, participants who responded “Strongly agree” were categorized as being able to smell asparagus and those who responded “Moderately agree,” “Slightly agree,” “Slightly disagree,” “Moderately disagree,” and “Strongly disagree” were categorized as having asparagus anosmia. Those who responded “I don’t eat asparagus” were excluded from the analysis.

**Statistical analysis**
We carried out multivariable logistic regression analyses, modeling single nucleotide polymorphisms as ordinal variables and asparagus anosmia as the outcome. Models were adjusted for age, sex, smoking status (never, former, and current), and the first three principal components of genetic variation (to adjust for potential confounding by ethnicity). The analyses were conducted separately for each of the three genotyping platforms and the estimates were combined using a fixed effects meta-analysis. We considered P values $<5\times10^{-8}$ to indicate genome wide significance, and all tests were two sided.

To further explore the association between genetic variation and asparagus anosmia, we performed sequential conditional analysis using GCTA-COJO, a tool for genome wide complex trait analysis. The supplementary file describes these analyses in detail. Briefly, this method allows adjustment for single nucleotide polymorphism-B when evaluating the association between single nucleotide polymorphism-A and anosmia to determine if they have independent effects or are both associated with the outcome through correlation.

We then used the variant effect predictor tool to determine the effect of the variants on the amino acid sequence. Using the PolyPhen analysis tool (version 2.2.2, http://genetics.bwh.harvard.edu/pph2/), we explored the possible impact of missense mutations (ie, a change in one DNA base pair that results in the change in one amino acid for another in the protein) on the structure and function of a protein. PolyPhen uses a set algorithm to predict the likelihood that the single nucleotide polymorphism will be “probably damaging” (that is, high confidence the single nucleotide polymorphism affects protein function or structure), “possibly damaging” (supposed to affect protein function or structure), or “benign” (most likely lacking any phenotypic effect).

**Patient involvement**
No patients were involved in setting the research question or the outcome measures, nor were they involved in developing plans for recruitment, design, or implementation of the study. No patients were asked to advise on interpretation or writing up of results. There are no plans to disseminate the results of the research to study participants or the relevant patient community.

**Results**
Among 6909 participants, 39.8% (n=2748) strongly agreed that they could perceive a distinct odor in their urine after eating asparagus and 60.3% (n=4161) said they could not and were classified as asparagus anosmic (table 1). The proportion of participants unable to detect the odor was slightly lower among men in the Health Professionals Follow-up Study compared with women in the Nurses’ Health Study.

Overall, 871 single nucleotide polymorphisms reached genome wide significance ($P<5\times10^{-8}$) for asparagus anosmia (see supplementary table 1). Figure 1 displays a Manhattan plot in which each dot represents a single nucleotide polymorphism laid out across the chromosomes from left to right. The height of the peaks corresponds to the strength of association with asparagus anosmia. The large peak represents a 0.46 Mb region on chromosome 1 (248139851-248595299). This region was split into two subregions by a recombination hotspot (see supplementary fig 2) and contained multiple members of the olfactory receptor 2 (OR2) gene family. The single nucleotide polymorphism identified previously in one study and validated in another study (rs481887) was also significantly associated with asparagus anosmia in this population ($P=1.4\times10^{-43}$) and is located in the same 1q44 region identified in this analysis.

Sequential conditional analysis revealed three loci independently associated with asparagus anosmia in this region (rs13373863, rs71538191, and rs6689553) (table 2). After conditioning on these three single nucleotide polymorphisms, no other single nucleotide polymorphism reached genome wide significance. Supplementary tables 2 and 3 and supplementary figs 2a and 2b present a more detailed analysis and visualization of this region.

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Table 1 | Characteristics of study population by ability to smell metabolites of asparagus in urine, 2010. Values are numbers (percentages) unless stated otherwise

| Characteristics | HPFS (n=2500) | NHS (n=4409) |
|-----------------|--------------|--------------|
|                 | Anosmic | Able to smell | Anosmic | Able to smell |
| Study sample    | 1449 (58)   | 1051 (42)    | 2712 (62) | 1697 (38)    |
| Mean (SD) age (years) | 76 (8) | 75 (8) | 78 (6) | 76 (6) |
| Smoking status  |             |              |            |              |
| Current smoker  | 50 (2)      | 25 (1)       | 221 (5)   | 132 (3)      |
| Former smoker   | 1100 (44)   | 1100 (44)    | 2160 (49) | 2160 (49)    |
| Never smoker    | 1370 (54)   | 1375 (55)    | 2028 (46) | 2116 (48)    |

HPFS=Health Professionals Follow-up Study, NHS=Nurses’ Health Study.
Polymorphisms from the 1000 Genomes Project, which receptor 2 gene family.
some 1, containing multiple members of the olfactory genome wide significance. All were located on chromo-
association studies with the anosmia trait, we found 871 in their urine. Linking information from genome wide and women, three in five were unable to detect the odor common. In this study of 6909 European-American men
Anosmia for the urinary metabolites of asparagus is
Discussion
Anosmia for the urinary metabolites of asparagus is common. In this study of 6909 European-American men and women, three in five were unable to detect the odor in their urine. Linking information from genome wide association studies with the anosmia trait, we found 871 unique single nucleotide polymorphisms reaching genome wide significance. All were located on chromosome 1, containing multiple members of the olfactory receptor 2 gene family.
Our analyses included imputed single nucleotide polymorphisms from the 1000 Genomes Project, which allowed us to more thoroughly identify novel single nucleotide polymorphisms that might interact with the previously identified single nucleotide polymorphism to produce the anosmic phenotype. The previous genome wide association studies of asparagus anosmia identified an association with rs4481887, 8993 base pairs upstream of OR2M7. By incorporating comprehensive efforts to refine the signal, we identified three independent association signals in this region, tagged by rs13373863, rs71538191, and rs6689553. Although we did not conduct a further replication study of the genome wide signals, our findings validate and extend the previously reported associations between OR2 and asparagus anosmia. Both our study and a previous study were conducted on people of European descent; thus it is unclear whether results would differ in non-European populations.

Two “probably damaging” missense single nucleotide polymorphisms in OR2M7 in strong linkage disequilibrium with rs6689553 (rs7555310 and rs7555424) are intriguing candidate variants that might be responsible for this association. We also identified a missense variant in OR2L3 that could be responsible for the association signal tagged by rs13373863. OR2L3, OR14C36, and OR2M7 are thought to be involved in G-protein receptor and olfactory receptor activity, and OR14C36 in the binding of an odorant to its receptor. The molecular basis at the root of human olfaction is not fully understood. Research has investigated specific anosmias and hyperosmias as a key to understanding olfaction, often focusing on the genetic determinants of these phenomena to better understand the overall functional relation. Our findings present candidate genes of interest for future research on the structure and function of olfactory receptors and on the compounds responsible for the distinctive odor produced by asparagus metabolites. Answering these questions might shed light more generally on the relation between the molecular structure of an odorant and its perceived odor.

Asparagus has long been recognized as a delicacy. Writings by the Roman scholar Cato the Elder around 200 BC provide a method of planting asparagus,15 and a recipe for cooking asparagus is found in Apicius’s De Re Coquinaria from the late 4th century AD.16 According to

### Table 2 | Results of stepwise conditional analysis: single nucleotide polymorphisms (SNPs) that remained independently statistically significantly associated with asparagus anosmia after mutual adjustment

| SNP                  | Reference allele | Alternate allele | Frequency of reference allele | Marginal odds ratio (95% CI) | Marginal P value | Conditional odds ratio (95% CI) | Conditional P value |
|----------------------|------------------|------------------|-----------------------------|-----------------------------|----------------|---------------------------|----------------------|
| rs13373863           | A                | G                | 0.07                        | 1.13 (1.08 to 1.17)          | 4.13e-10       | 1.15 (1.11 to 1.20)         | 5.51e-14             |
| rs71538191           | C                | G                | 0.60                        | 0.85 (0.84 to 0.87)          | 1.86e-41       | 0.90 (0.89 to 0.92)         | 7.02e-13             |
| rs6689553            | T                | C                | 0.32                        | 1.15 (1.13 to 1.17)          | 4.26e-44       | 1.11 (1.08 to 1.13)         | 7.51e-19             |

### Table 3 | Missense single nucleotide polymorphisms (SNPs) associated with asparagus anosmia at genome wide significance (P<5×10⁻⁸)

| SNP                  | Gene | Possible impact | PolyPhen score | Reference allele | Alternate allele | Marginal odds ratio (95% CI) | Marginal P value |
|----------------------|------|-----------------|----------------|-----------------|-----------------|-----------------------------|----------------|
| rs6658227            | OR2L3| Probably damaging| 0.97           | T               | C               | 1.07 (1.05 to 1.09)          | 2.94e-9         |
| rs28545014           | OR14C36| Probably damaging| 1              | T               | G               | 0.93 (0.91 to 0.95)          | 3.53e-12        |
| rs71538191           | OR2M7| Probably damaging| 0.97           | A               | G               | 1.14 (1.12 to 1.16)          | 2.62e-43        |
| rs7555424            | OR2M7| Possibly damaging| 0.85           | A               | G               | 1.14 (1.12 to 1.16)          | 2.50e-43        |
the United States Department of Agriculture food composition database, asparagus is rich in iron, fiber, zinc, folate, and vitamins A, E and C. Consumption of asparagus, as part of a diet high in vegetables, has been hypothesized to reduce the risk of cancer, cognitive impairment, and cardiovascular related diseases. Under the assumption that the urinary odor from asparagus consumption leads to avoidance of this vegetable, future work should consider Mendelian randomization studies using these identified single nucleotide polymorphisms to better understand how a lifetime of eating asparagus might protect people from developing chronic conditions. Furthermore, literature from the 15th century touts its aphrodisiac qualities. Studies could investigate assortative mating with respect to asparagus anosmia; however, differentiating choice of partner based on preference for flavor or odor would be challenging.

Interestingly, women in the Nurses’ Health Study were more likely to report asparagus anosmia than men in the Health Professionals Follow-up Study, despite the fact that women have been shown to more accurately and consistently identify smells. We hypothesize that this unexpected result might be due to underreporting by a few modest women who are loath to admit they can smell the distinctive odor in their urine. It is possible that women are less likely than men to notice an unusual odor in their urine because their position during urination might reduce their exposure to volatile odors. This highlights a weakness of our study design that is shared by many studies of olfactory perception—namely, that we rely on self report of perception rather than on an objective measurement of olfactory stimulation. Our study is also limited by a one-time measure of anosmia; therefore, we do not have information on whether the ability to smell asparagus metabolites changes with age, and previous studies have shown that as humans age, their general olfactory function declines.

Outstanding questions on this topic remain; first and foremost perhaps is why a delicacy such as asparagus results in such a strong odor? Why does genetic variation across the olfactory receptor genes exist that leads to susceptibility to asparagus anosmia; the non-anosmic wish to remain anonymous. However, the first and last authors admit they can both produce and detect the “filthy and disagreeable smell in the urine.”

Ethical approval: The study protocols were approved by the institutional review boards of Harvard TH Chan School of Public Health and Partners Healthcare.

Data sharing: Data from the genome wide association studies are provided on dbGAP (www.ncbi.nlm.nih.gov/gap), a database on genotypes and phenotypes.

Transparency: The lead author (LAM) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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**Supplementary information:** supplemental methods

**Supplementary table:** supplemental tables 1-5

**Supplementary figure:** QQ plot to show distribution of the observed and expected P values of the different platforms used to generate the genetic data: (a) Affymetrix platform, (b) Illumina, and (c) combination of both platforms