Responses to Editor and Reviewer Comments:

This is a good research question for Christmas and the title is engaging. But the rest of the paper is dense and difficult for generalists to follow.You probably overestimate our readers knowledge of genetic research and genome wide association studies. When you re draft please consider enlisting the help of someone with absolutely no expertise in this area- medical students are good for this. If they can understand what you have done, and why it matters, then our readers should have no problem with the revision. This is particularly important in headline parts of the paper [abstract, discussion, what this paper adds box]

More specifically: Please explain what you mean by "mis sense" variant and why are they referred to as "damaging".

The figures are poorly explained and won't mean much to our readers. Perhaps select one [Eg the Manhattan plot] and explain fully. Consider putting the others in an appendix

Thank you for your consideration of our work for the Christmas issue. We have revised the manuscript to make the analyses and results more clear, including explaining the terms missense variants and 'damaging' in the statistical analysis section. We have also moved more detailed descriptions of the methods and results to the Appendix. Additionally, we have included only one figure (Manhattan plot) with an extended description in the body of the text.

Please don't overshoot the 2000 word limit, or cutting for print will be extremely difficult.

We have cut down the text of the manuscript to <2000 words and can be further cut if necessary.

This is serious research but in the spirit of Christmas you should consider adding some light relief, possibly in the form of a table listing famous people who can (or can't) smell asparagus metabolites in urine. You already mention Benjamin Franklin [please add the quote and a citation], and Proust [Asparagus transforms my chamber pot into a flask of perfume." Proust https://thesproutdiaries.com/2011/05/23/asparagus-transforms-my-chamber-pot-into-a-flask-of-perfume/]. Editors and advisors at the meeting also mentioned the Roman writer Cato in his book,De re rustica' [unverified] and the Scottish mathematician Arbuthnot [http://www.bbc.com/future/story/20140818-mystery-of-asparagus-and-urine].

We have added some additional light relief to the body of the manuscript, primarily in the introduction and discussion sections of the paper, including a discussion of future research based on these findings.

Thank you for alerting us to the Cato reference. We have added this and some additional ancient texts to a discussion about the benefits of asparagus.

Can all authors smell these metabolites? [possible addition to competing interest

statement]

We have added a fun fact about our asparagus ansomia to the competing interest statement, and in the study population section of the manuscript.

Reviewer 1 questions the narrow focus of your work. But the consensus at the meeting was that this was fine for a Christmas paper on one specific aspect of smell.
Based on the consensus at the meeting, we will keep the focus of the paper on asparagus anosmia.

REVIEWERS COMMENTS:
Reviewer: 1
The paper is well written, but I don't see how it fits into the overall Christmas journal spirit apart from the title. There are issues here regarding the dataset and overall perspective of the paper. It would seem that focusing on a large number of individual genes, no doubt correlated, without understanding of the functions involved or how the phenotype in question, clearly important, can be narrowed to a specific individual smell.

We have added some additional light relief to the introduction and discussion sections of the paper, and moved some of the detailed methods and results to supplementary material.

Based on the consensus at the meeting, we kept the focus of the paper specifically on GWAS of asparagus anosmia, but discussed that "*OR2L3*, *OR14C36*, and *OR2M7* are thought to be involved in G-protein receptor and olfactory receptor activity, and *OR14C36* in odorant binding." We agree that the molecular basis of smell is complicated, and state that "…Answering these questions may more generally shed light on the relationship between the molecular structure of an odorant and its perceived odor."

• Is the article important?
• Will it help our readers to make better decisions and, if so, how?
• Will the article add enough to existing knowledge?

The article, for the Christmas edition of BMJ, has an amusing title.
The p-values as reported for the various gene expressions seem appropriately determined and interpreted. Multiple comparisons and GWAS methods are applied.

We appreciate this comment, and have tried to make the body of paper more in the Christmas journal spirit.

• Does the article read well and make sense? Does it have a clear message?

Yes it has a clear but limited message.
It is clearly written.

For research articles

• Originality — does the work add enough to what is already in the published literature? If so, what does it add? Please cite relevant references to support your comments on originality.

The study is a standard application of GWAS methods to a specific table item found on many Christmas tables. That said it has a serious tone.

We appreciate this comment, and have tried to make the body of paper more in the Christmas journal spirit, including a discussion of asparagus on the holiday table – thank you for this suggestion.

• Importance of the work to general readers — does this work matter to clinicians, researchers, policymakers, educators, or patients? Will it help our readers to make better decisions and, if so, how? Is a general medical journal the right place for it?

It may be of interest to general readership.

We have edited the manuscript to hopefully read more clearly to a general audience and have moved detailed analyses and results to the Appendix for those wishing to see them.

That said it is not particularly insightful. The focus on asparagus only is somewhat questionable. A keen sense of smell is a phenotypic quality that may correlate across many smells. Why should there be a distinct asparagus gene. Might it be a more general olfactory related gene? Is there any evidence that asparagus can be isolated in this manner.

We agree that the molecular basis at the root of human olfaction is not fully understood. To address this point, we discuss that “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 relationship.”

In addition, our more detailed analysis found SNPs in three olfactory related genes *OR2L3*, *OR14C36*, and *OR2M7*, which are all thought to be involved in G-protein receptor and olfactory receptor activity, and *OR14C36* in odorant binding. We have added this information to the discussion.

We have hopefully better addressed these points in the discussion on page 12.

There is no focused discussion of possible correlates.
The main objective of this paper was a GWAS study of asparagus anosmia.

The dataset has a high number of non-sensitive olfactory subjects, including a surprisingly high number of females. If the olfactory phenotype is known to segregate on gender, why is this not seen in this dataset. What is unique to the asparagus sensing related genes so they do not display this result? They should not be distinct from the general olfactory related genotype-phenotype relationship. This may imply possible aggregation issues or bias in the dataset.

o Overall design of study — appropriate and adequate to answer the research question?

There seem to be difficulties in the sense that a key gender segregation does not occur.

We are unaware on any previous research evaluating gender differences specifically for asparagus ansomia, and are unaware of any previous results on potential gene-environment interactions with this phenotype. Previous research as shown that women potentially have a better sense of smell – a study from Brazil found that women have more cells in the olfactory bulb, the area of the brain dedicated to the sense of smell; however, these results are not specifically related to asparagus. In our study, the difference in asparagus anosmia between men and women was small (58% vs 62%), and when re-categorized anosmia as strongly or moderately agree (as discussed in the appendix), the proportion of anosmic was similar between men and women (46% vs 47%). Furthermore, differences in prevalence of non-sensitivity by gender do not imply there are gene-gender interactions, and even if other evidence suggests there are gene-gender interactions on average or at specific loci, that does not imply these loci have to exhibit gene-gender interactions.

While we could've had misclassification of our phenotype, which could decrease our power, this would not induce bias. Given we see such a strong signal, in a region that makes sense biologically and replicates previous results, bias is an unlikely explanation for our findings. We do discuss some potential limitations of our study on page 12 of the discussion.

Also the focus on asparagus in relation to isolating genes seems questionable as a conclusion.

Based on the consensus at the meeting, the focus on one aspect of smell, asparagus, seems appropriate for the Christmas edition.

o Participants — adequately described, their conditions defined, inclusion and exclusion criteria described? How representative were they of patients whom this evidence might affect?

The participants and the obtaining of samples is clearly defined.

They reflect specific populations.

o Methods — adequately described?

Yes.

o Main outcome measure clear?

Yes.

o Was the study ethical (this may go beyond simply whether the study was approved by an ethics committee or IRB)?

Yes.

o Results — answer the research question? Credible? Well presented?
o Interpretation and conclusions — warranted by and sufficiently derived from/focused on the data? Discussed in the light of previous evidence? Message clear?

The genetics of smell, olfactory, are obviously important. Perhaps more important in past settings where food safety was always a serious issue. A keen and accurate sense of smell would no doubt prove protective and an element of phenotypic fitness. It would have evolved as one of the key human senses. It would have been constantly used in many different settings. Thus the genetics of olfactory would not necessarily be focused on specific foods but rather the underlying chemical basis of foods, which are found in varying amounts across a wide range of plants and vegetables (and other foods). I am not sure it makes clear sense to analyze one particular result using genetics.

As mentioned above, the molecular basis at the root of human olfaction is not fully understood, and we agree that there are likely multiple compounds in asparagus that contribute to the odor.

Given the number of genes found to be significant here and their presentation in terms of ranked p-values (note the ranking would surely alter to some extent under replication give the very small absolute values being observed) there is probably an argument for a gene network or gene networks expressing in a correlated manner to guide the phenotype of "sense of smell". This can be analyzed using correlated networks and might be more relevant here.

We agree that the ranking of the p-values might be slightly different if conducted in a further replication set. However, our validation of the previous finding by Ericksson and Pelchat, and our more in-depth evaluation of the signals through fine-mapping strengthen our findings. Based on the comments at the consensus meeting and for the purposes of the Christmas issue, correlated networks may be too complicated for the general readership.

o References — up to date and relevant? Any glaring omissions?

The references seem acceptable. As genetic analysis is always improving and related methods of statistical analysis adjusting, there is some leeway to be given. The use of p-values in a GWAS context seems standard.

We attempted to include the most up to date and relevant references.

o Abstract/summary/key messages/what this paper adds — reflect accurately what the paper says?

Yes, the abstract is appropriate.

o Supplemental: Do these properly match what is in the manuscript? Do they contain information that should be better reported in the manuscript, or raise questions about the work?

The supplemental materials match what is in the manuscript. Note the use of the "expected p-values" wording is a bit questionable though standard, as the p-values in these settings are clearly based on actual observations. We have no expectation of what the p-value will be. Rather it is the p-value calculated under a specific assumed context that is referenced. As such reporting all of them in tabular forms and then again in the graphs might be more than what is required. Hard to believe in clear rankings when comparing 10 to the minus 42 versus 10 to the minus 43.

That said reporting actual p-values and letting the scientist or reader interpret them within the specific scientific context is what Fisher suggested many times.

We agree and report the actual p-values obtained from the GWAS analysis. We included all of the SNPs that reached genome-wide significance ($p<5x10^{-8}$) in Supplementary Table 1 for readers to have full access to all of the rs numbers for the statistically significant SNPs and their associations with asparagus anosmia.

Conclusion

I believe the paper is too narrow and the data seem to have some challenges. A discussion of the results in relation to the more general issue of the olfactory phenotype is lacking.

Apart from the title, I don't see a Christmas spirit or sense of humor in the presentation.

We appreciate this comment, and have tried to make the body of paper more in the Christmas journal spirit. We have edited the manuscript to hopefully read more clearly to a general audience and have moved detailed analyses and results to the Appendix for those wishing to see them.

Reviewer: 2
General comments

In this interesting (and somewhat peculiar) study, the authors performed a GWAS to uncover novel genetic loci associated with one's ability to smell the odor produced after eating asparagus (i.e., asparagus anosmia). Only one previous GWAS, conducted in the customer base of 23andMe, has examined this common trait—identifying an association with rs4481887 (near OR2M7 in 1q44). The present study identified 871 variants reaching genome-wide statistical significance, all located in the same locus (1q44) as that reported by Eriksson et al. Although the authors did not identify novel genetic loci related to asparagus anosmia, these data add to the limited literature by performing extensive analyses that identify 3 independent association signals in this previously identified region, one of which was strongly correlated with the previous GWAS variant. In addition, based on bioinformatics-based functional data, the authors suggested potential causal variants warranting follow-up.

This study has several strengths, including the relatively large population pulled from well-characterized cohorts. Importantly, the authors should be commended

for their comprehensive follow-up efforts to refine and characterize association signals—including extensive conditional analyses as well as fine-mapping. The manuscript is clear and easy to read.

There are a few questions/concerns that should be addressed before this manuscript is ready for publication.

Specific comments

1) This study does not include a conventional replication step. Because of the regularity of false positive findings in GWAS, even after applying the stringent significance threshold, I expected to see replication of the genome-wide signals, particularly because this study included only two European cohorts. It is unclear if the authors forgo replication here because the significant signals were in a previously identified region with known olfactory function? Or because they are being validated to a certain extent in subsequent analyses and fine-mapping? Regardless, the authors should provide strong rationale for why replication was not conducted, and acknowledge the potential limitations of such a design.

We agree that replication serves two important functions: first, to establish that the finding represents an association truly present in the sampled population(s); and second, to demonstrate that the association is not the result of some idiosyncrasy of the sampled population or study design bias [PMID: 20454541]. The fact that we exceeded the already conservative GWAS significance of $5x10^{-8}$ by many orders of magnitude demonstrates that we have achieved the first goal of replication; our observed association is almost certainly not due to chance. We believe we have satisfied the second goal both by seeing consistent effects across two distinct cohorts (representing different demographic groups, including, most notably, different genders), and by replicating a previously-published finding. As you mention, because we validated and extended the previous findings, we did not conduct a further replication study. In addition, we thought it would add complication for the purposes of the BMJ Christmas issue.

On page 10 in the discussion, we address this point:
“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.”

2) Along these lines, it would be worthwhile to note as a limitation that this study as well as that conducted by Eriksson et al. was conducted in individuals of European descent. It is thus unclear whether the frequency/genetic predictors of asparagus anosmia would differ in non-European populations.
We agree and have added this point to this discussion on page 10:
“Both our study and Eriksson *et al* were conducted on individuals of European descent; thus, it is unclear whether results would differ in non-European populations.”

3) What is the rationale for restricting functional follow-up only to coding variants in strong LD with any of the independent lead markers (and the results additionally only focus on missense variants)? As non-coding SNPs in strong LD with the lead markers could be critical in gene regulation, restricting to coding variants could be missing potential causal variants. Accordingly, it might also be

worth performing functional annotation of non-coding variants (e.g., ENCODE, eQTL analysis, etc.).

We focused on nonsynonymous coding SNPs both because nsSNPs directly implicate candidate genes and because several lines of evidence suggest that coding SNPs show greater enrichment for genetic association than regulatory SNPs [PMID: 25439723; PMID: 26414678] (although in total regulatory SNPs account for more of the genetic variance than coding SNPs, because more SNPs fall in regulatory than coding regions). We did explore the potential links between the top independent SNPs in Table 2 and regulatory variation, but found no clear links. We now include the following sentence in the results in the Appendix:

"We also explored whether the three SNPs in Table 2 that tag the three independent signals at 1q44 were in strong linkage disequilibrium with SNPs in regulatory regions or known eQTLs using HaploReg and the GTEx portal. None of these SNPs had an r2>0.8 with any known eQTL or a SNP in promoter histone marker, enhancer histone markers or DNAse hypersensitive regions."

4) The authors note that the 3 variants established to be independent based on sequential conditional analysis were correlated at r2<0.7. I understand these signals are "independent" based on conditional p<5E-08. However, based on my understanding that r2=0.7 is a moderately strong correlation, these variants don't seem entirely "independent." It would be helpful if the authors provided more discussion of how likely these signals are truly "independent" vs. the low conditional p-value being a function of the very strong marginal associations (consistent with this, the conditional betas for rs71538191 and rs6689553 are weaker in conditional analyses). For instance, it would be informative to include the pairwise correlation values between these 3 variants in Table 2.
Thank you for this suggestion. The LD between these SNPs is small. In the Appendix on page 4 we have added the pairwise correlation between each of them:
"These three SNPs are not in strong LD with each other (r^2 rs13373863-rs71538191 = 0.002; r^2 rs13373863-rs6689553 = 0.01; r^2 rs71538191-rs6689553 = 0.43)."

Minor comments

1) Abstract
a. To help interpretation, please note all 871 statistically significant SNPs were located in 1q44.
Thank you for this suggestion. We have added this to the results in the abstract:
"…all in a region on chromosome 1 (1q44: 248139851-248595299)."

b. Please include allele frequencies and corresponding gene(s) for the 3 independent markers.
For general audiences it might be unclear what the frequency refers to without being able to explain this, therefore, we have not included the allele frequencies in the abstract, but they are included in Table 2. We can add them to the abstract if the reviewers and editors feel it is necessary.

We added in the results that the genome-wide significant SNPs are in a region containing multiple genes in the *OR2* family:

"871 SNPs 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."

c. Would be helpful if the Results noted the relationship between these 3 markers and the previous GWAS finding. (As a reader, my first question was whether these 3 markers were independent of the previously identified GWAS variant.)
We have added the following information to address this point:
"Conditional analyses revealed three independent, including independent of the SNP previously identified (rs4481887), markers associated with asparagus anosmia: rs13373863, rs71538191, and rs6689553."

2) Methods
a. How many variants were tested in total?
There were about 9 million variants that passed QC thresholds included in the study. We have included this information in the appendix in the statistical genotyping paragraph (page 2).

b. Why was smoking an adjustment factor? Age, sex, and PCs are self-explanatory, but smoking is not.
We included smoking as a covariate because previous research has shown that smoking is associated with a reduced ability to smell.

3) Results
a. Please be explicit that the SNP previously identified by Eriksson et al. is also located in the 1q44 region identified in this analysis.
Thank you for this suggestion. We have added this to the results page 7:
"The SNP previously identified by Eriksson *et al* and validated by Pelchat *et al* (rs4481887) was also significantly associated with asparagus anosmia in this population (p-value 1.41×10^{-43}) and is located in the same 1q44 region identified in this analysis."

b. Betas are difficult to interpret for a lay reader. It would be helpful if the authors present in a separate table the %'s of those who can vs. cannot smell the odor across genotypes for each of the 3 independent variants.
We have turned the betas and SEs into odds ratios and 95% confidence intervals in Tables 2 and 3, which should be more interpretable to the general readership.

In addition, we have added the percentages of those who can and cannot smell the odor across the genotypes for the 3 variants in Supplemental Table 3. If the reviewers and editors would prefer this to be in the primary tables, we can add do that instead.

c. Table 2

i. Were associations with these variants examined separately in men and women? Please include a heterogeneity p-value for these results across sex.

We have added the heterogeneity chi-square and p-values for each of the three SNPs from the combined analysis into the results and Supplemental Table 2 in the Appendix. The Cochran's Q p-values from the combined analysis show no significant variation.

We have conducted the marginal analyses for the 3 SNPs separately for men and women and find attenuated, but similar results:

MALES:

SNP	N Can Smell	N Anosmic	Reference Allele	Alt Allele	Frequency of Reference Allele	Marginal OR	Marginal 95% CI	Marginal p-value
rs13373863	1051	1449	A	G	0.06	1.10	(1.03-1.17)	0.0027
rs71538191	1051	1449	C	G	0.59	0.87	(0.84-0.91)	8.25e-12
rs6689553	1051	1449	T	C	0.32	1.12	(1.08-1.16)	6.39e-12

FEMALES:

SNP	N Can Smell	N Anosmic	Reference Allele	Alt Allele	Frequency of Reference Allele	Marginal OR	Marginal 95% CI	Marginal p-value
rs13373863	1697	2712	A	G	0.07	1.14	(1.09-1.19)	2.38e-8
rs71538191	1697	2712	C	G	0.59	0.84	(0.81-0.86)	8.83e-32
rs6689553	1697	2712	T	C	0.31	1.16	(1.14-1.19)	1.45e-34

We can add this information into the main body of the manuscript or supplemental material if the editors and reviewers would like.

ii. For clarity, please include the locus and corresponding gene(s) for each SNP.
We have added this information into Supplemental Table 2.

iii. Please include conditional p-value for these 3 SNPs.
The conditional p-values have been added to Table 2 as requested.

iv. As mentioned above, would be helpful to have pairwise correlations between these variants in table or text.
We have added this information into the appendix (page 4).

v. Were all 3 SNPs imputed in both NHS and HPFS? If so, please include the imputation Rsq.
Yes, all three SNPs were imputed. The Rsq for the Affymetrix and Illumina platforms are included below. We have also added this information to the Appendix results section (page 4).

SNP	Rsq Affymetrix	RsqIllumina
rs13373863	0.81	0.81
rs71538191	0.50	0.39
rs6689553	0.81	0.73

4) Discussion
a. Is it possible to calculate % variation of asparagus anosmia explained by the statistically significant variants?

We have analyzed anosmia as a binary trait, using logistic regression. 'Percent variance explained' by exposures are not routinely reported for binary traits. Measures such as Nagelkerke's R^2 could be reported, however these are difficult to interpret and hard to compare across studies or phenotypes, as they depend on prevalence in the analyzed sample. For genetic variants, the percentage of liability or familial relative risk explained is sometimes presented. In general, three SNPs with modest effects do not discriminate well between cases and controls, suggesting that anosmia is a complex, multifactoral trait, as we have mentioned in the discussion. If the reviewers and editors would like, we can add the results of a genetic risk score combining these SNPs to show discrimination between those subjects with and without anosmia; however, this may be too dense for the general readership.

b. Add a line or two briefly describing the function of OR2L3, OR14C36, and OR2M7.
In the discussion on page 12/13 we have added the following sentence:
"*OR2L3*, *OR14C36*, and *OR2M7* are thought to be involved in G-protein receptor and olfactory receptor activity, and *OR14C36* in odorant binding."

c. Is there any data to suggest that asparagus anosmia might change as an individual ages? I don't know the answer to this question, but this would affect the definition of the outcome.
To our knowledge, there is no research on asparagus anosmia itself, but studies do suggest that an individual's ability to smell decreases as they age.

In the discussion on page 12 we address this as a potential limitation of our study:

*“*Our study is also limited by a one-time measure of ansomia; therefore, we do not have information on whether the ability to smell asparagus metabolites changes with age. Studies have shown that as we get older, our olfactory function declines.”