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## Citrus-Gene Interaction and Melanoma Risk in the UK Biobank

Andrew R. Marley<sup>1</sup>, Ming Li<sup>2</sup>, Victoria L. Champion<sup>3,4</sup>, Yiqing Song<sup>1</sup>, Jiali Han<sup>\*,1,4</sup>, Xin Li<sup>\*,1,4</sup>

<sup>1</sup>Department of Epidemiology, Indiana University Richard M. Fairbanks School of Public Health, Indianapolis, USA

<sup>2</sup>Department of Epidemiology and Biostatistics, Indiana University School of Public Health, Bloomington, USA

<sup>3</sup>Department of Community Health Systems, Indiana University School of Nursing, Indianapolis, USA

<sup>4</sup>Indiana University Melvin and Bren Simon Comprehensive Cancer Center, Indianapolis, USA

### Abstract

High citrus consumption may increase melanoma risk; however, little is known about the biological mechanisms of this association, or whether it is modified by genetic variants. We conducted a genome-wide analysis of gene-citrus consumption interactions on melanoma risk among 1,563 melanoma cases and 193,296 controls from the UK Biobank. Both the 2-degrees-of-freedom (df) joint test of genetic main effect and gene-environment (G-E) interaction and the standard 1-df G-E interaction test were performed. Three index SNPs (lowest p-value SNP among highly correlated variants [ $r^2 > 0.6$ ]) were identified from among the 365 genome-wide significant 2-df test results (rs183783391 on chromosome 3 [*MITF*], rs869329 on chromosome 9 [*MTAP*], rs11446223 on chromosome 16 [*DEF8*]). Although all three were statistically significant for the 2-df test (4.25e-08, 1.98e-10, and 4.93e-13, respectively), none showed evidence of interaction according to the 1-df test (p=0.73, 0.24, 0.12, respectively). Eight non-index, 2-df test significant SNPs on chromosome 16 were significant (p<.05) according to the 1-df test, providing evidence of citrus-gene interaction. Seven of these SNPs were mapped to *AFG3L1P* (rs199600347, rs111822773, rs113178244, rs3803683, rs73283867, rs78800020, rs73283871), and one SNP was mapped to *GAS8* (rs74583214). We identified several genetic loci that may elucidate the association between citrus consumption and melanoma risk. Further studies are needed to confirm these findings.

**Corresponding author:** Dr. Xin Li, Department of Epidemiology, Indiana University Richard, M. Fairbanks School of Public Health, Health Sciences Building, RG 5114, 1050 Wishard Blvd, Indianapolis, IN, 46202, USA, Phone: +1 (317) 274-4538, XL16@iu.edu.  
\*Drs. X. Li, J. Han equally contributed as senior authors

#### Conflict of Interest

The authors declare no competing interests.

#### Ethics statement

The current study is exempt ethical/institutional approval as it is a secondary analysis of previously collected, deidentified data and authors made no attempts to re-identify or contact study participants.

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## Keywords

citrus; melanoma; GWAS; gene-environment interaction

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## Introduction

Incidence of melanoma, the most deadly form of skin cancer, is increasing faster than any other cancer.<sup>1,2</sup> The rapid observed growth in melanoma incidence is not artifactual,<sup>3</sup> and with global rates increasing by 3–7% per year,<sup>2</sup> incidence is doubling every 10–20 years.<sup>4</sup> Melanoma is also associated with considerable mortality. In the US, there are projected to be 7,180 melanoma deaths in 2021,<sup>5</sup> and, in the UK, melanoma mortality has increased by 144% since the early 1970s.<sup>6</sup> Globally, melanoma is the fastest growing cause of cancer death save for non-Hodgkin's lymphoma, lung cancer in women, and testicular cancer.<sup>1</sup>

The etiology of melanoma is multifactorial, involving environmental risk factors, genetic variants, and the interactions between them. High citrus consumption is an environmental factor that has received increased attention in recent years due to citrus products' natural abundance of psoralen, a type of furocoumarin known to be photosensitizing and photocarcinogenic<sup>7</sup> in mice<sup>8,9</sup> and in humans.<sup>10,11</sup> Research to this end has yielded inconsistent findings, as results from the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS)<sup>12</sup> have demonstrated an overall positive association between citrus consumption and melanoma risk, and findings from the Women's Health Initiative (WHI)<sup>13</sup> and the European Prospective Investigation into Cancer and Nutrition (EPIC)<sup>14</sup> have suggested positive associations between citrus consumption and melanoma risk among those who spent the most time outdoors in summer and those with the highest consumption of citrus fruit, respectively. Our own previous research in the UK Biobank (UKBB) has also suggested a positive association, finding a 63% increased risk of melanoma among participants in the highest category of total citrus intake.<sup>15</sup> The inconsistency of these findings is possibly due to heterogeneity of study populations and/or differences in methods of quantifying citrus consumption. None of these studies, however, have investigated the role of possible nutrient-gene interaction. It has also been established that an individual's genotype may influence melanoma risk. Previous candidate gene studies and genome-wide association studies (GWAS) have identified several genes that are associated with melanoma risk,<sup>16,17</sup> but there is currently no evidence as to whether genetic variation modulates the association between melanoma risk and citrus consumption.

As it is critical to identify nutrient-gene interactions that may influence the etiology and pathophysiology of melanoma, the purpose of the current study was to conduct, to our knowledge, the first genome-wide analysis of citrus-gene interactions on risk of melanoma. We believe the results of the current study will increase knowledge of melanoma pathology, identify genetic variants and gene-environment (G-E) interactions underlying photocarcinogenesis in melanoma, and, upon further validation, could serve as an empirical basis for the development of a skin cancer prediction model, eventually leading to improved precision prevention.

## Methods

### Data Collection, Genotyping, Imputation, and Quality Control

A more detailed description of UKBB genomic methodology is described elsewhere.<sup>18</sup> The UKBB is a large, prospective cohort with extensive genomic data collected for all approximately 500,000 participants. Participants were all from the UK and aged 40–69 years at recruitment between 2006–2010. Each participant was assessed at one of 22 assessment centers throughout the UK and provided blood samples from which DNA was extracted and sent to Affymetrix Research Services Laboratory for genotyping. Upon receipt at Affymetrix, samples were then processed on the GeneTitan Multi-Channel Instrument in 96-well plates containing 94 UKBB samples and two control samples from the 1000 Genomes Project. Genotyping was carried out in 106 batches of approximately 4,700 samples. The first 50,000 participants were genotyped on the UK BiLEVE Axiom Array, and the following 450,000 participants were genotyped using the UK Biobank Axiom Array. Although different arrays were used, they share 95% of the same marker content, and only markers present on both arrays were used. Autosomal phasing was performed using SHAPEIT3, with the 1000 Genomes phase 3 dataset as the reference panel. The UKBB was also imputed using merged UK10K and 1000 Genomes phase 3 reference panels. Principal components (PCs) were computed via an algorithm in which 407,219 high quality, unrelated samples and 147,604 high quality markers were used and pruned to minimize linkage disequilibrium.

Poor quality markers were identified using statistical tests to check for consistency of genotype calling. These included tests for batch effects, plate effects, departures from Hardy-Weinberg equilibrium, sex effects, array effects, and discordance across control replicates. Markers failing at least one test in a batch had the genotype calls in that batch set as missing, and if a marker was not reliable across all batches, it was excluded altogether. Markers were also removed if they had at least a 5% overall missing rate or if they had a minor allele frequency <0.0001.

Poor quality samples were identified using missing rate and heterozygosity that were computed using 605,876 high quality autosomal markers typed on both arrays. Samples that were outliers for heterozygosity or missing rate were removed, as were a small number of samples identified as duplicates and approximately 10 samples that were mishandled in the laboratory. Overall, these filters and exclusions resulted in a dataset with 93,095,623 autosomal SNPs, short indels and large structural variants in 487,442 samples.

### Citrus Intake, Melanoma Ascertainment, and Measurement of Covariates

Citrus consumption in the UKBB were collected via five ‘rounds’ of 24-hour recall questionnaires. The five ‘rounds’ took place between April 2009–September 2010, February–April 2011, June–September 2011, October–December 2011, and April–June 2012, respectively, with the first round taking place in the assessment center and subsequent rounds administered electronically. This electronic 24-hour recall yielded a mean Spearman’s correlation coefficient of 0.6 (range 0.5–0.9) compared with an interviewer-assisted 24-hr recall.<sup>19</sup> Although collection of citrus consumption data was more recent,

we are confident using it as a proxy for past citrus consumption in the UK due to data from other wealthy nations suggesting that average fruit intake is staying consistent over time,<sup>20,21</sup> and due to 82% of UKBB cases reporting the same or adjacent category of fresh fruit consumption between the first and last dietary assessments.<sup>22</sup> A total of n=210,126 participants completed at least one 24-hour dietary recall to provide complete citrus consumption data. Consumption of orange, grapefruit, satsuma, orange juice, and grapefruit juice were categorized into four intake groups: 0, half, >half-1, and >1 serving. A cumulative average of this citrus consumption over the 5 ‘rounds’ of nutritional data collection was used to assess total citrus consumption. For this cumulative average, total citrus consumption was categorized into the following groups: ‘none’, ‘>0-half a serving’, ‘half-1 serving’, ‘>1–2 servings’, ‘>2 servings’. Participants were not required to complete all five ‘rounds’ of data collection for inclusion in the analysis, and any untaken ‘rounds’ were treated as missing and therefore had no impact on participants’ cumulative average for citrus consumption. ICD codes (C43.0–9) were used to identify melanoma outcome data. Cases were acquired via these codes, which were linked with national registries that obtain cancer diagnosis data from various sources (such as hospitals, nursing homes, death certificates, general practices, etc.) and retrospectively dated back to the early 1970s. The current study focused on melanomas of the skin and case ascertainment was not limited to primary melanoma cases. Age of the participants was derived based on date of birth provided at the assessment center. It refers to participants’ age when they first visited the center, truncated to whole year. A participant’s sex was acquired from the National Health Service at recruitment, and, in some cases, updated by the participant. Due to its noted strength as a melanoma risk factor,<sup>23</sup> tanning ability was also included as a covariate. Tanning ability was measured by asking “What would happen to your skin if it was repeatedly exposed to bright sunlight without any protection?”, with the following as our coded responses for this variable: “get very tanned”, “get moderately tanned”, “get mildly or occasionally tanned”, and “never tan, only burn”. There were no missing data for participants’ age or sex, and missing data for tanning ability was not substantial (1.6%), therefore it was simply excluded from the analysis.

### Statistical Analysis

Of the n=210,126 with complete citrus data, n=11,162 non-Caucasian participants (5.3%) were excluded as done in previous analyses<sup>12,13</sup> due to low melanoma incidence in ethnic minorities.<sup>24</sup> Genetic data for another n=4,105 (2.0%) were also excluded due to quality control procedures, leaving n=194,859 Caucasian UKBB participants available for this analysis. From this sample, we included SNPs with a minor allele frequency >0.01 and an imputation quality score >0.3. After applying these filters, a total of 9,981,017 SNPs were analyzed in this study.

We created a quantile-quantile (QQ) plot and calculated lambda ( $\lambda$ ) to assess genomic inflation. Interactions between citrus consumption and genetic variants were evaluated by performing the joint 2-degrees-of-freedom (df) test of genetic main effect and G-E interaction. The 2-df joint test performed a test of SNP marginal effects and their interaction with citrus consumption by conducting likelihood ratio tests between the full logistic regression model ( $\text{Logit}(\text{Pr}(\text{melanoma}=1)) = b_0 + b_1 * \text{total citrus consumption} + b_2 * \text{SNP}$

+  $b_3$ \*total citrus consumption\*SNP +  $b_4$ \*age +  $b_5$ \*gender +  $b_6$ \*tanning ability +  $b_7$ \*PC1+ ... +  $b_{21}$ \*PC15) and the reduced model (excluding SNP and total citrus consumption\*SNP). This approach has been found to be much more powerful than the standard test and better suited for discovering new genetic markers and investigating new potential G-E interactions, making it a valuable tool for large discovery scans in which true gene-environment associations are unknown.<sup>25,26</sup> Of the significant results ( $p < 5e-08$ ), one index SNP was selected (SNP with the lowest p-value) from among highly correlated variants ( $r^2 > 0.6$ ).

Next, to gauge whether the joint 2-df test results were being primarily driven by interaction or genetic main effect, we also performed the 1-df likelihood ratio test comparing the above full model vs. the model without the multiplicative interaction term. This test was performed on all significant SNPs from the 2-df test. SAS version 9.4 (SAS Institute, Cary, NC), PLINK version 2.0 ([www.cog-genomics.org/plink/2.0/](http://www.cog-genomics.org/plink/2.0/)), and R version 3.6.0 (The R Foundation for Statistical Computing, Vienna, Austria) were used for the described analyses. All data storage and programming were performed in Karst, a high-performance computing cluster at Indiana University.

## Results

Our analyses were conducted on a total of 194,859 Caucasian UKBB participants, including 1,563 cases and 193,296 controls. As illustrated in Figure 1, the genomic variants in our analysis have a fairly normal chi-squared distribution with little evidence of genomic inflation ( $\lambda = 1.031$ ). After adjusting for age, gender, tanning ability, and the first 15 PCs, the 2-df joint test revealed a total of 365 SNPs that were significant at a p-value  $< 5e-08$  (Table S1), including one on chromosome three, 270 on chromosome nine, and 94 on chromosome 16. Of these, 3 index SNPs were identified: rs183783391 ( $p = 4.25e-08$ ) on chromosome 3 (3p13), rs869329 ( $p = 1.98e-10$ ) on chromosome 9 (9p21.3), and rs11446223 ( $p = 4.93e-13$ ) on chromosome 16 (16q24.3) (Table 1). These three SNPs are mapped to *MITF*, *MTAP*, and *DEF8*, respectively. Although p-values for these index SNPs reached statistical significance for the joint test, neither rs183783391 ( $p = 0.73$ ), rs869329 ( $p = 0.24$ ), or rs11446223 ( $p = 0.12$ ) showed evidence of interaction with citrus consumption according to the conventional 1-df test. Evidence of interaction ( $p < 0.05$ ) was observed for 8 of the 365 SNPs found to be significant ( $p < 5e-08$ ) by the 2-df joint test (Table 2). These SNPs, all on chromosome 16, included rs199600347, rs111822773, rs113178244, rs3803683, rs73283867, rs78800020, and rs73283871 mapped to *AFG3L1P* and rs74583214 mapped to *GAS8*.

## Discussion

Although noted for challenges and some inconsistent/inconclusive results,<sup>27,28</sup> genome-wide studies to identify G-E interactions influential to common traits and cancer are of critical importance and some have provided evidence of environment-associated genetic effects.<sup>29-31</sup> Although previous epidemiological evidence has suggested that high citrus consumption may increase melanoma risk,<sup>12-15</sup> little is known regarding the genetics of citrus metabolism, and the potential role of genetic variants involved in citrus-associated melanoma risk had been previously unexplored. In the current analysis, we tested the hypothesis that the increased risk of melanoma associated with high citrus consumption is an

effect of G-E interaction. We identified three index SNPs that were highly significant for the joint 2-df test: rs183783391 (*MITF*), rs869329 (*MTAP*), and rs11446223 (*DEF8*).

Mapped to *MITF* on chromosome 3 (position 69950451), rs183783391 is an intronic variant located between the first and second exon. This SNP is not within a conserved motif or associated with any *MITF* regulatory elements. Other melanoma susceptibility variants have also been identified on chromosome 3, including rs3950296 mapped to *TERC* and rs149617956 mapped to the p.E318K functional variant on *MITF*,<sup>32,33</sup> however, these variants are not in linkage disequilibrium with rs183783391 ( $r^2 = 0.2$ ). Located at 3p13, *MITF* (microphthalmia-associated transcription factor) plays a major role in the development, function, and survival of pigment-producing melanocytes.<sup>34,35</sup> As melanoma tumors are derived from melanocytes, *MITF* has been recognized for its role in driving melanoma progression and has been shown to regulate senescence, differentiation, proliferation, apoptosis, and migration of melanoma cells.<sup>35,36</sup> Additionally, due to its role in the transformation of immortalized melanocytes and its expression in conjunction with *BRAF*<sup>V600E</sup>, *MITF* demonstrates oncogenic properties.<sup>37,38</sup> Genetic epidemiology studies have also linked *MITF* with melanoma risk. Previous research has elucidated the role of *MITF* in human pigmentation,<sup>32,39</sup> a known melanoma risk factor.<sup>23</sup> Additional evidence has directly linked *MITF* with human melanoma risk, highlighting its role in familial melanoma.<sup>40</sup> Therefore, although rare, we hypothesize that rs183783391 may play a role in melanoma etiology via its association with one of these mechanisms.

Mapped to *MTAP* on chromosome 9, rs869329 is an intronic variant at position 21804693 between the first and second exon. This SNP is not within a conserved motif of the genome or within any regulatory elements. Many melanoma susceptibility variants have been previously identified on chromosome 9, with particularly strong associations mapped to *CDKN2A*,<sup>32,41,42</sup> a gene linked to an estimated 40% of familial melanoma cases.<sup>43</sup> As *MTAP* is in close physical proximity to *CDKN2A* and rs869329 is in high linkage disequilibrium with previously identified *CDKN2A* SNPs ( $r^2=0.76-1.00$ ), it is possible that rs869329 serves as a *CDKN2A* tag. However, *MTAP* and *CDKN2A* are frequently co-deleted, causing *MTAP* loss to often be attributed to *CDKN2A*,<sup>44</sup> and evidence has suggested that *MTAP* may have tumor suppressor function independent of *CDKN2A*.<sup>45,46</sup> Previous genome-wide research has also found melanoma risk to be linked with *MTAP*, whether directly,<sup>47</sup> or through its association with cutaneous nevi,<sup>44,48,49</sup> another known melanoma risk factor.<sup>50</sup> Additionally, a study by McMeniman et al. specifically found that single primary melanoma patients with melanoma at a site of visible UV-damage were significantly more likely to carry rs869329 relative to controls (odds ratio = 1.4 [CI=1.1-1.7]).<sup>51</sup> *MTAP* (methylthioadenosin phosphorylase), located at 9p21.3, is critical to polyamine metabolism and the salvage of adenine and methionine. It also acts in catalyzing the phosphorylation of methylthioadenosine, which plays a role in the inhibition of methyltransferases and polyamine aminopropyltransferase.<sup>46</sup> Although typically expressed in cells and tissues, malignant cells tend to have decreased *MTAP* and have been shown to secrete methylthioadenosine rather than metabolize it.<sup>45,46</sup> Because of this, we hypothesize that rs869329 influences melanoma risk independent of *CDKN2A*.

Our final index SNP, rs11446223, is an intronic variant at position 90022484. This SNP is not within any regulatory element and can be mapped to *DEF8* on chromosome 16. Current knowledge is limited regarding the precise role and function of *DEF8* (differentially expressed in FDCP 8 homolog) located at 16q24.3. *DEF8* encodes an activator of intracellular signal transduction,<sup>52</sup> however, as it is located just downstream of rs1805007 on *MC1R* (linkage disequilibrium  $r^2 = 0.41$ ), a melanoma-susceptibility gene known to play a role in skin pigmentation and sun sensitivity,<sup>53</sup> it is unclear whether this signal is due to *MC1R* proximity or whether *DEF8* has a more prominent independent role.<sup>54</sup> Although likely this SNP tags for *MC1R*, a recent publication described a significant association between *DEF8* and melanoma risk,<sup>55</sup> demonstrating the possibility that *DEF8* may have independent melanoma-susceptibility properties.

Although, these genes have been previously found to be associated with melanoma risk, none of the index SNPs identified by the 2-df joint test were significant for the standard 1-df test, indicating that these results are being primarily driven by the genetic main effects rather than citrus consumption or citrus-gene interaction. However, testing the remaining significant 2-df test significant SNPs revealed a possible G-E interaction between citrus consumption melanoma risk for eight SNPs on chromosome 16, including seven SNPs on the pseudogene gene *AFG3LIP* and one on *GAS8*. Of the seven *AFG3LIP* SNPs, rs199600347, rs111822773, rs113178244 are intronic variants, rs3803683 is a non-coding transcript variant, rs73283871 is a downstream transcript variant, and rs73283867 and rs78800020 are each split with two variants downstream of the gene and two non-coding transcripts. Most rs74583214 (*GAS8*) transcripts indicated 3 prime UTR variants, and the SNP is located within *MYC* and *POLR2A* elements.

A couple of possibilities exist that could explain this evidence of interaction with *AFG3LIP*. Firstly, although these signals were strong for *AFG3LIP*, it is possible that this gene may not represent the true susceptibility variants, but rather tag *MC1R* within the same locus for which there is a biologically plausible association with melanoma<sup>56</sup> (e.g, with the exception of rs3803683 [ $r^2 = 0.39$ ], all our *AFG3LIP* SNPs are in high linkage disequilibrium with rs1805007, an *MC1R* locus strongly associated with melanoma risk<sup>32</sup> [ $r^2 = 0.61-0.95$ ]). Furocoumarins have been demonstrated to reach peak concentration in the skin within four hours of oral intake and remain detectable in cutaneous tissue for at least seven hours after intake.<sup>57</sup> These furocoumarins, when exposed to UV radiation, can cause the formation of reactive oxygen species (ROS) and thereby induce photo-oxidative damage by accessing epidermal, dermal, and endothelial cells.<sup>57,58</sup> UV exposure to furocoumarin-sensitized skin can also modify proteins, inactivate enzymes, and produce mutagenic and carcinogenic effects.<sup>59,60</sup> Therefore, it is biologically plausible to observe an interaction between furocoumarins obtained through citrus intake and *MC1R*, which influences pigment metabolism and the ability to protect against UV radiation<sup>61</sup> and also plays a role in the growth and development of melanoma cells via its influence on keratinocyte and melanocyte proliferation and differentiation.<sup>62,63</sup>

Secondly, it is also possible for *AFG3LIP* to play an independent role in this association. Although pseudogenes are typically superfluous and nonfunctional, evidence suggests that some of these genes are translated, and therefore may play a meaningful, functional role in

human biology.<sup>64</sup> Evidence from Bánfai et al. suggests that *AFG3LIP* is one of these rare exceptions, indicating it is transcribed and appears to be translated.<sup>65</sup> Furthermore, Bánfai et al. conclusively mapped a consistently detected peptide to a novel exon downstream of the pseudogene transcriptional unit that is both beyond the parental gene similarity region and absent in the parental gene locus.<sup>65</sup> This research provides meaningful support of *AFG3LIP* having a novel, protein-coding function distinct from the parental gene,<sup>65</sup> and possibly having an independent function in the association between citrus consumption and melanoma risk. Although further validation studies are needed, *AFG3LIP* may possibly play a previously unknown role in citrus metabolism, photocarcinogenesis, or psoralen/furocoumarin absorption.

The interaction we found with rs74583214 on *GAS8* may influence melanoma risk via similar mechanisms. *GAS8* is a tumor suppressor gene that has been linked to several types of cancer,<sup>66–69</sup> including melanoma.<sup>70,71</sup> As *GAS8* has been implicated in melanoma risk via its influence on pigmentation,<sup>71</sup> furocoumarin exposure may exacerbate UV sensitivity due to *GAS8* expression. Also, a rs7458314 is in strong linkage disequilibrium with rs1805007 on *MC1R* ( $r^2=0.71$ ), it is also possible that rs74583214 serves as a tag for *MC1R*, which also plays a significant role in pigmentation.<sup>61</sup>

Recent work by Landi et al. identified 86 genetic variants implicated in melanoma susceptibility.<sup>32</sup> We further evaluated if the effect of these genetic variants may be modified by citrus consumption. Eight variants were found with potential gene-citrus interaction in our analysis (Table S2). Details for this additional analysis as well as variants found significant according to the 2-df test, and their associated 1-df test results, can be found in the supplementary material.

As with all studies, our study is subject to limitations. As with all environmental variables, possible population-to-population or country-to-country variance in citrus consumption may limit our results to this specific British cohort. We are limited by our use of recent citrus consumption as a proxy for past citrus consumption. We were also limited by our inability to control for personal or family melanoma history. Additionally, it is possible that our sample size (1,563 melanoma cases included in the analysis) may not be large enough to detect modest effects. Lastly, our analyses and estimates are based on common SNPs, and it is therefore plausible that rare variants could have contributed meaningful data to this analysis.

In conclusion, in this genome-wide analysis, we found that rs183783391 (*MITF*), rs869329 (*MTAP*), and rs11446223 (*DEF8*) are significantly associated with melanoma according to the joint 2-df test; however, 1-df tests found no evidence of citrus-gene interaction, demonstrating that these findings are primarily driven by gene main effects, and not by citrus consumption. Further analyses revealed that seven SNPs on *AFG3LIP* and one SNP on *GAS8*, which could possibly serve as tags for *MC1R* or have independent citrus metabolism or psoralen absorption effects, were significant on both the joint and standard tests, giving evidence of possible citrus-gene interaction.



## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Data Availability Statement

This work has been conducted using the UK Biobank Resource under Application Number 49419. The UK Biobank is an open access resource and bona fide researchers can apply to use the UK Biobank dataset by registering and applying at <http://ukbiobank.ac.uk/register-apply/>. Further information is available from the corresponding author upon request.

## Abbreviations:

<b>df</b>	degrees-of-freedom
<b>EPIC</b>	European Prospective Investigation into Cancer and Nutrition
<b>G-E</b>	gene-environment
<b>GWAS</b>	genome-wide association studies
<b>HPFS</b>	Health Professionals Follow-up Study
<b>NHS</b>	Nurses' Health Study
<b>PC</b>	principal components
<b>QQ</b>	quantile-quantile
<b>UKBB</b>	UK Biobank
<b>WHI</b>	Women's Health Initiative

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**Novelty and Impact:**

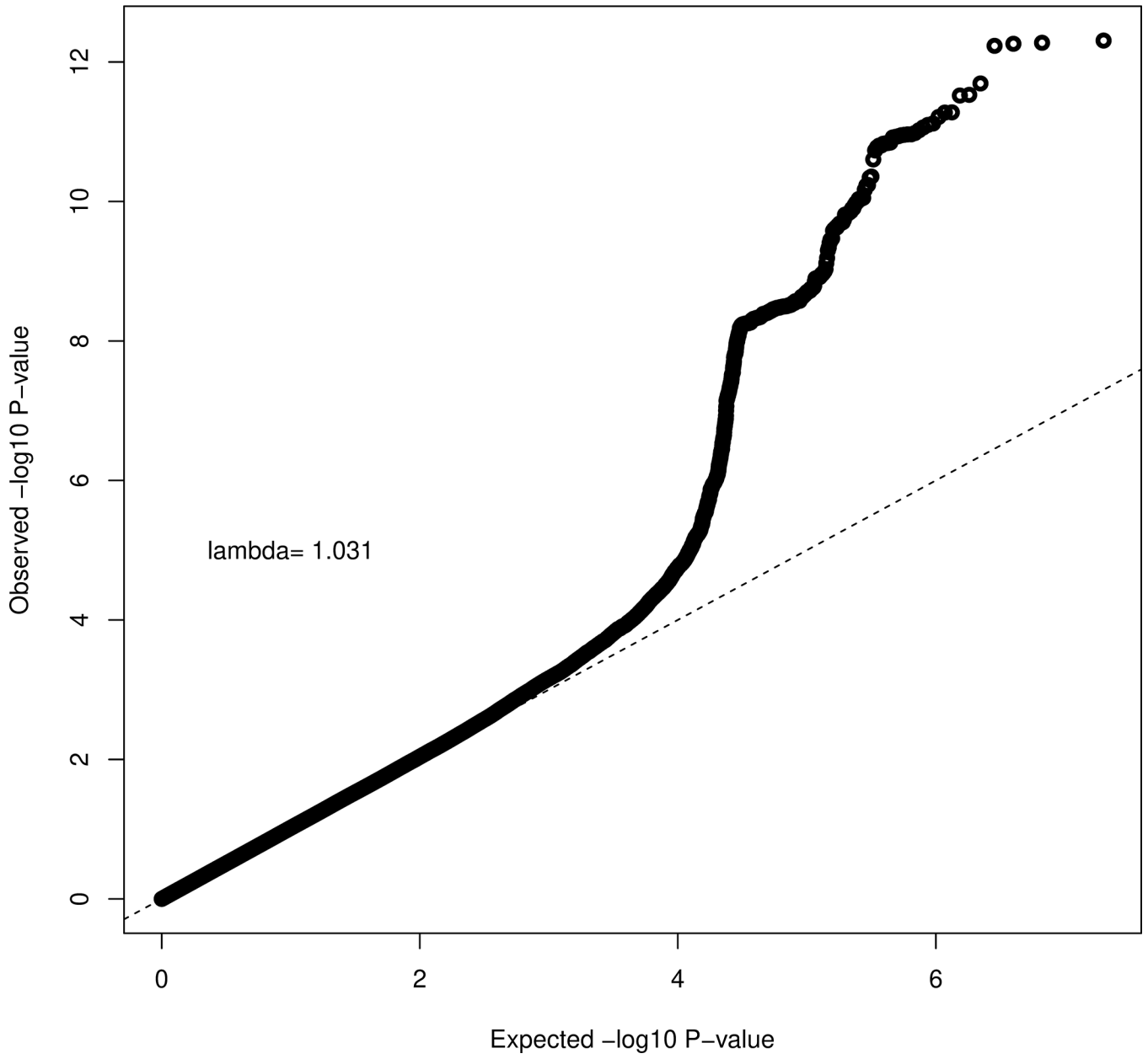
Previous research has suggested an association between high citrus consumption and melanoma risk. Although inconsistent, findings from these large cohorts were based on the biologically plausible hypothesis that high consumption of citrus increases risk of melanoma due to photocarcinogenic psoralen and furocoumarins that are naturally abundant in citrus products. None of this research has investigated possible genetic bases for this association or identified genetic variants that may modify it. Here, authors conduct a novel genome-wide gene-environment interaction analysis, identifying several genetic loci that provide evidence of citrus-gene interaction for melanoma risk. These findings suggest a genetic basis for photosensitivity and photocarcinogenicity and could advance melanoma precision prevention.

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**Figure 1.**  
QQplot of Joint 2DF Test across 22 Chromosomes

Index single nucleotide polymorphisms (SNPs) resulting from a genome-wide analysis of gene-citrus consumption interaction on melanoma risk.

**Table 1.**

SNP ID	Chr	Position	Region	Associated gene	Minor allele	MAF	Info score	p-2DF	p-IDF
rs183783391	3	69950451	3p13	<i>MITF</i>	T	0.013	0.95	4.25e-08	0.73
rs869329	9	21804693	9p21.3	<i>MTAP</i>	A	0.48	0.99	1.98e-10	0.24
rs11446223	16	90022484	16q24.3	<i>DEF8</i>	GA	0.17	0.99	4.93e-13	0.12

Abbreviations: Chr, chromosome; Ref allele, reference allele; MAF, minor allele frequency; Info score, imputation quality score; p-IDF, p-value for the conventional 1 degree-of-freedom test; p-2DF, p-value for the 2 degree-of-freedom joint test



**Table 2.** Non-index SNPs significant for the joint 2 degrees-of-freedom test and with evidence of citrus-gene interaction.

SNP ID	Chr	Position	Region	Closest gene	Minor allele	MAF	Info score	p-2DF	p-1DF
rs199600347	16	90052245	16q24.3	<i>AFG3L1P</i>	TTA	0.10	0.91	3.53e-10	0.023
rs111822773	16	90054089	16q24.3	<i>AFG3L1P</i>	C	0.11	0.98	3.62e-09	0.043
rs113178244	16	90056195	16q24.3	<i>AFG3L1P</i>	G	0.11	0.99	5.00e-09	0.049
rs3803683	16	90060281	16q24.3	<i>AFG3L1P</i>	C	0.17	0.99	3.55e-10	0.047
rs73283867	16	90066260	16q24.3	<i>AFG3L1P</i>	G	0.096	1.00	9.54e-12	0.050
rs78800020	16	90067136	16q24.3	<i>AFG3L1P</i>	C	0.096	1.00	1.12e-11	0.045
rs73283871	16	90067202	16q24.3	<i>AFG3L1P</i>	C	0.096	1.00	1.09e-11	0.045
rs74583214	16	90110798	16q24.3	<i>GAS8</i>	T	0.098	0.96	5.57e-09	0.043

Abbreviations: Chr, chromosome; Ref allele, reference allele; MAF, minor allele frequency; Info score, imputation quality score; p-1DF, p-value for the conventional 1 degree-of-freedom test; p-2DF, p-value for the 2 degree-of-freedom joint test

Note: all SNPs are in strong linkage disequilibrium with each other ( $R^2$  0.60–1)