# Individuals with *CYP2C8* and *CYP2C9* reduced metabolism haplotypes self-adjusted ibuprofen dose in the Coriell Personalized Medicine Collaborative

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**Objectives** The objectives of this study were to determine whether differences in *CYP2C8* and *CYP2C9* haplotype influence the dose of ibuprofen self-administered by individuals, and to examine the potential relationship between *CYP2C8* and *CYP2C9* reduced metabolism haplotypes and adverse events.

**Participants and methods** We investigated relationships between genetic variations in *CYP2C8* and *CYP2C9* and ibuprofen use, dose, and side effects (reported by questionnaire) in 445 participants from the Coriell Personalized Medicine Collaborative.

**Results** Carriers of reduced metabolism haplotypes for *CYP2C8* (*2, *3, *4) and *CYP2C9* (*2, *3) were significantly ($P = 0.0171$) more likely than those lacking these variants to take less than the recommended dose of ibuprofen, after controlling for sex, age, race, and cohort. In contrast to ibuprofen dose, there were no differences in ibuprofen use frequency or reported side effects based on haplotype. However, there are often no early signs of acute kidney injury, the most serious side effect of elevated ibuprofen exposure.

**Conclusion** These results suggest a subset of individuals with genetic variation in *CYP2C8* and *CYP2C9* recognize that they obtain adequate drug efficacy with lower ibuprofen doses, or take lower doses due to prior side effects. However, most (82.6%) individuals with reduced metabolism haplotypes nonetheless took recommended or higher doses, potentially putting them at increased risk for side effects. *Pharmacogenetics and Genomics* 00:000–000

## Introduction

Ibuprofen, a chiral NSAID, is available over-the-counter in many countries. In the USA, ibuprofen was approved for over-the-counter use in 1984 [1,2], and the maximum approved over-the-counter and prescription daily doses are 1200 and 3200 mg, respectively. Although commonly used and generally considered safe, NSAIDs are often used without physician supervision or knowledge [3], and are associated with substantial excess morbidity and mortality [4,5]. Ibuprofen is commonly used at high frequencies and doses by athletes for pain management and recovery, despite risks of side effects in this setting [6–8]. Ibuprofen is the most common medication used for treatment of noncombat injuries in theater in the US military, accounting for over 60% of pain medications received by service members deployed to Iraq or Afghanistan in 2003–2004 [9,10]; similarly, 27% of US soldiers deployed to Somalia in 1993–1994 received ibuprofen [11]. Side effects commonly reported for ibuprofen include hemorrhage, gastrointestinal pain, hearing loss, kidney problems, and hypersensitivity reactions [1,12,13].

Metabolism of ibuprofen occurs mainly, though not exclusively, by oxidative metabolism. Notable routes of nonoxidative metabolism are as follows: α-methylacyl-CoA racemase catalyzes the conversion of (*R*)-ibuprofen to its *S* enantiomer [14,15], and uridine 5′-diphospho-glucuronosyltransferases account for ∼ 15% of ibuprofen clearance [16].

Oxidative metabolism of ibuprofen occurs primarily by cytochrome P450 (CYP) 2C8 and 2C9 enzymes [17–20]. It has been reported that individuals with reduced

ibuprofen metabolism due to genetic variation in *CYP2C9* are at an increased risk for developing acute gastrointestinal bleeding after taking ibuprofen and other NSAIDs [21–23], demonstrating that differences in ibuprofen metabolism can influence side-effect risk. Similarly, it has been reported that the combined use of NSAIDs and selective serotonin uptake inhibitors, some of which are CYP2C9 inhibitors, can increase the risk for gastrointestinal side effects due to drug–drug interaction and/or pharmacogenetic variation [24–29]. Consistent with these observations, differences in *CYP2C8* and *CYP2C9* haplotypes (frequencies of which vary by ancestry [30]) have been associated with substantial changes in ibuprofen pharmacokinetics [31–34]. For example, ibuprofen clearance was reduced by an average of 35% in individuals with the common diplotypes *CYP2C8* *1/*3 and *CYP2C9* *1/*2 (compared with *CYP2C8* *1/*1 and *CYP2C9* *1/*1), and reductions in clearance of up to 10- and four-fold were observed for *CYP2C8* *3/*3 and *CYP2C9* *3/*3 diplotypes, respectively, compared with *1/*1 [35]. *CYP2C8* and *CYP2C9* haplotypes were also shown to influence the frequency of emergency department visits for pain management in an African–American sickle cell disease patient cohort [36]. On the basis of these observations in the literature, we conducted this analysis to test the hypothesis that differences in *CYP2C8* and *CYP2C9* haplotypes may influence the dose of ibuprofen self-administered by individuals, and to examine the potential relationship between *CYP2C8* and *CYP2C9* reduced metabolism haplotypes and adverse events.

## Participants and methods
### Samples
The Coriell Personalized Medicine Collaborative (CPMC) is an ongoing, prospective study designed to evaluate the utility of genomics in clinical decision-making and health management [37] in which participants receive personalized complex disease and pharmacogenomic reports [38,39]. Data from the study are also used for research, including GWAS [40] and behavioral studies [41,42]. At the time of this study, there were ~ 6000 active participants in the CPMC. For this ancillary study, 480 individuals were recruited and incentivized ($10) to provide information on their use of over-the-counter pain medications (specifically for pain relief and not other indications, e.g. daily low-dose aspirin).

The CPMC is comprised of several cohorts, and individuals from each were included in the current study [37,38]: a community cohort from the general population in the Delaware Valley ($n = 175$), a cancer cohort at Fox Chase Cancer Center ($n = 7$), a chronic disease cohort at Ohio State Medical Center ($n = 39$), a community cohort recruited through Ohio State University ($n = 39$), and an US Air Force cohort ($n = 198$). All participants were adults

($\geq 18$ years) who had given written informed consent. In total, data from 445 participants were included in the current study. The Coriell Institute Institutional Review Board has reviewed and approved protocols for each of the above-mentioned cohorts, and the Institutional Review Boards of Fox Chase Cancer Center, Virtua Health System, Ohio State University Medical Center, and the US Air Force have approved their respective cohort-specific protocols. This work was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013.

### Genotyping
Each participant previously provided a saliva sample from which DNA was extracted using the Oragene method (DNA Genotek Inc., Ottawa, Ontario, Canada) as part of their enrollment into the CPMC. Coriell's in-house Clinical Laboratory Improvement Amendments-certified Sequencing and Microarray Center [37,38] used the Affymetrix DMET Plus array to genotype 1931 drug-metabolism-related single-nucleotide polymorphisms (SNPs) in 225 genes [43]. In total, 1863 SNPs passed research quality control filters (no more than 10% missing data for any given marker) and were retained for further analysis. All individual samples with genetic data ($n = 445$) included in this analysis had at least 97% complete SNP data.

On the basis of characteristic SNPs for each star allele included on the DMET Plus array in the *CYP2C8* and *CYP2C9* genes, individuals were haplotyped for *CYP2C8* *1, *2, *3, and *4, and *CYP2C9* *1, *2, and *3, as defined in Supplemental Table 1 (Supplemental digital content 1, *http://links.lww.com/FPC/B337*). For *CYP2C8*, *1B and *1C haplotypes were treated as *1, as there is no evidence that these variants in the promoter region of *CYP2C8* influence ibuprofen metabolism activity [44]. Although the *4 [45], *5 [46], and *6 [47] alleles for *CYP2C9* result in complete loss of enzyme activity, the frequencies of these star alleles are very low. As a result, no individuals in the analysis data set ($N = 445$) carried the variants corresponding to *CYP2C9* *4, *5, or *6, and these star alleles were excluded from this analysis. All other rare star alleles for *CYP2C8* and *CYP2C9* were not determined in this study and were treated as *1.

Genetic variation in α-methylacyl-CoA racemase, uridine 5′-diphospho-glucuronosyltransferases, or other non-CYP enzymes contributing to minor routes of ibuprofen elimination was not assessed in this study.

### Nongenetic data collection
CPMC participants use a secure web-based portal [37,38] to provide information related to medical history, medication use, family history, lifestyle, and demographics. This portal was also used to collect additional information on over-the-counter pain medication use by a short, voluntary, incentivized survey made available to all study participants. Participation was capped at 500 individuals. The complete survey is provided for reference as

Supplementary Appendix A (Supplemental digital content 2, *http://links.lww.com/FPC/B338*). Participants answered questions on daily aspirin use for reasons other than pain management, the frequency of use of different over-the-counter pain medications, medications preferred for different types of pain, typical doses taken, motivations for medication preference, and side effects associated with each medication. Pain severity was not assessed in this survey. The medications included were aspirin, ibuprofen, naproxen, acetaminophen, Excedrin, Anacin, and Midol. The specific question on medication dose was, 'For each medication, please indicate the typical dose you take to relieve pain. If you rarely (annually or less) or never take the listed medication, please select 'not applicable', with 'Less than the package recommends,' 'The dose recommended on the package,' 'More than the package recommends,' 'Not applicable,' or 'Don't Know' as choices.' While this question does not specify or ask for the exact dose of each medication, individuals often do not know the dose in milligrams they take of over-the-counter medications [48]. As a result, surveys of over-the-counter medication use often ask whether individuals take the recommended amount, or more or less [49], as was done here.

## Statistical methods

The two hypotheses tested in this analysis were whether *CYP2C8* and *CYP2C9* reduced metabolism haplotypes were associated with higher or lower self-administered doses of ibuprofen. A generalized linear model was used to test these hypotheses, with the number of *CYPs* with at least one reduced metabolism haplotype as the independent variable of interest (with possible values 0, 1, or 2), and greater or less than recommended ibuprofen dosing as the dependent variable. To account for the potential influence of demographic variables on ibuprofen dosing, sex, age, race, and study cohort were included as covariates [50], so that the *glm* call used in R was of the following form: $dose \sim no\_red\_cyps + gender + age + race + cohort$, wherein $no\_red\_cyps$ was the number of *CYPs* with at least one reduced metabolism haplotype (i.e. 0 if both *CYP2C8* and *CYP2C9* were *1/*1, and 1 or 2 if one or both, respectively, of the enzymes was heterozygous or homozygous for a reduced metabolism haplotype).

To control for multiple hypothesis testing, the Holm–Bonferroni procedure [51] was used to adjust the alpha threshold for significance for the two tested hypotheses. As an additional complementary analysis, the generalized linear model described above was also used to evaluate the relationship between the combined number of reduced metabolism haplotypes of either *CYP2C8* or *CYP2C9* (with possible values ranging from 0 to 4) and greater or less than recommended ibuprofen dosing.

In addition to the primary statistical analysis described above, the effects of *CYP2C8* and *CYP2C9* haplotypes and metabolism status (characterized in greater specificity, e.g. at the level of star alleles) on ibuprofen use, dose, and side effects (including abdominal pain, gastrointestinal symptoms, allergic reactions, as well as either any reported side effect or the total number of reported side effects) were explored using tests of equal proportion (i.e. *Z*-tests). The function *prop.test* in the base R statistics package was used to compute tests and provide *P* values (all *P* values from tests of equal proportion presented are two sided). Odds ratios were estimated from $2 \times 2$ contingency tables.

## Results

Of the 445 participants, 280 (62.9%) were women and 165 (37.1%) were men. The median (minimum–maximum) age of participants was 48 (21–79) years, and participants were primarily of European ancestry; 414 (93.0%) participants reported 'Caucasian' race. Most of the participants were from either the community [175 (39.3%)] or Air Force [198 (44.5%)] cohorts of the CPMC study. These demographics are similar to those of the broader CPMC cohort [40].

Ibuprofen was the most popular over-the-counter pain medication used by participants in the survey. A total of 351 (78.9%) individuals reported taking ibuprofen for pain yearly or more frequently, and 127 (28.5%) took it weekly or more frequently. As illustrated by the histogram in Fig. 1, ibuprofen was most commonly reported as taken at a weekly or monthly frequency, although ~ 20% of individuals reported never taking it, and 22 (4.9%) reported taking it daily or more than once daily. Further, ibuprofen was the leading over-the-counter pain medication for all categories of pain surveyed, including headache, menstrual cramps, joint pain, and back pain. As shown in Fig. 2, the primary motivations for taking ibuprofen among study participants were as follows: (i) it provided the best pain relief, (ii) it was the pain medication they have always taken, and (iii) it was recommended by a doctor.

Distributions of *CYP2C8* and *CYP2C9* haplotypes among study participants are presented in Fig. 3. Tables 1–3 show the relationships between drug metabolism haplotype and ibuprofen dose, at an increasingly granular level with respect to *CYP2C8* and *CYP2C9* haplotypes. As shown in Table 1, after controlling for sex, age, race, and study cohort using a generalized linear model, individuals with a greater number of *CYPs* with at least one reduced metabolism haplotype (i.e. carriers of *CYP2C8* *2, *3, *4 or *CYP2C9* *2, *3) were significantly (*P* = 0.0171, compared with a Holm–Bonferroni adjusted threshold of 0.025) more likely to report taking less than the recommended dose of ibuprofen, although individuals with fewer *CYPs* with at least one reduced metabolism haplotype were not less likely (*P* = 0.958, compared with 0.05) to report taking more than the recommended dose. Individuals with reduced metabolism haplotypes for both

**Fig. 1**
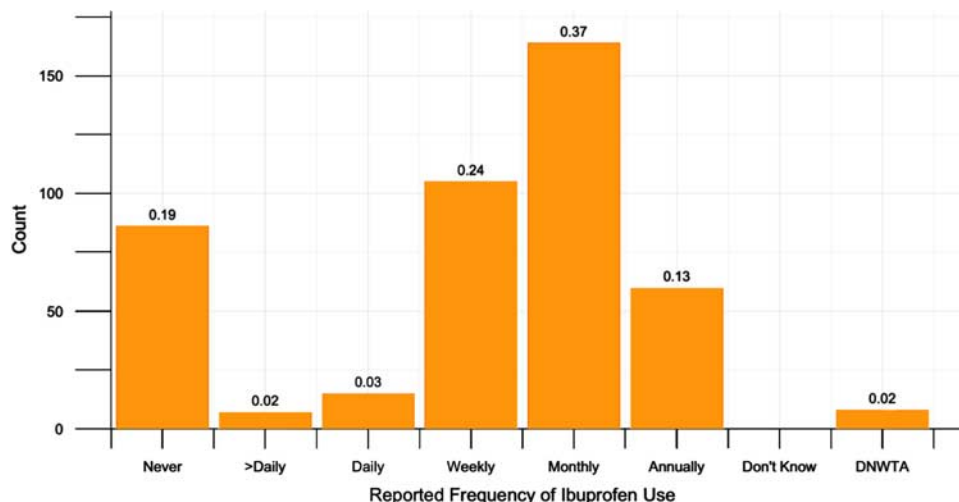


Ibuprofen use frequency (labels indicate the fraction of total). DNWTA, do not want to answer.

**Fig. 2**
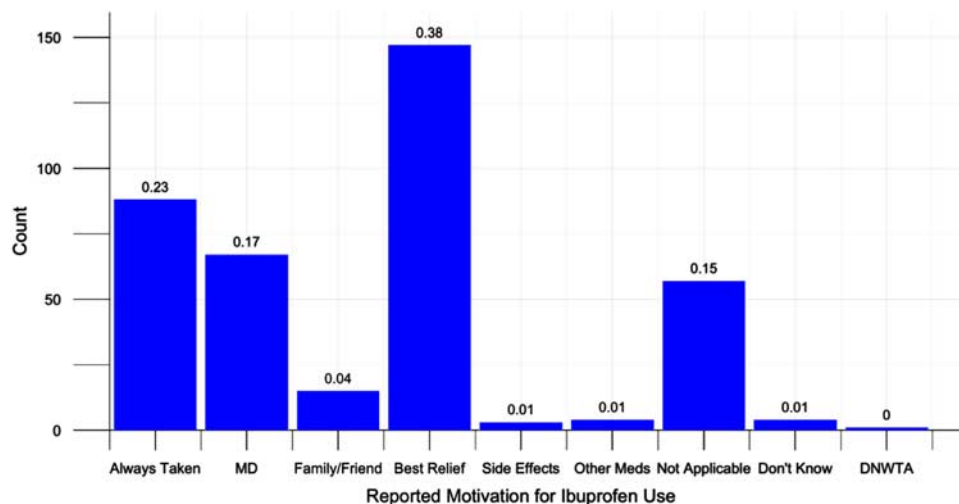


Primary motivation for ibuprofen use (labels indicate the fraction of total). DNWTA, do not want to answer; MD and family/friends, recommendation by those individuals; other meds, ibuprofen does not interact with other medications used; side effects, ibuprofen has a better side-effect profile than other options.

*CYP2C8* and *CYP2C9* were approximately four times more likely to take a lower than recommended dose of ibuprofen: 14.5% of these individuals took less than the recommended dose, compared with 4.4 and 2.6%, respectively, of individuals with zero or one *CYP* with at least one reduced metabolism haplotype. None of the covariates tested (sex, age, race, and study cohort) was significantly associated with taking less or more than the recommended dose of ibuprofen. That said, the observed association of reduced metabolism haplotype with an increased likelihood of taking a lower ibuprofen dose was driven primarily by women: eight of 10 individuals with reduced metabolism haplotypes taking less than the recommended dose of ibuprofen were women, although, as noted above, over 60% of participants in this study were women.

When the generalized linear model used to evaluate this relationship was modified to treat the combined number of reduced metabolism haplotypes of either *CYP2C8* or *CYP2C9* (range: 0–4) as the independent variable, the association with taking less than the recommended dose of ibuprofen remained, although its significance was

## Fig. 3
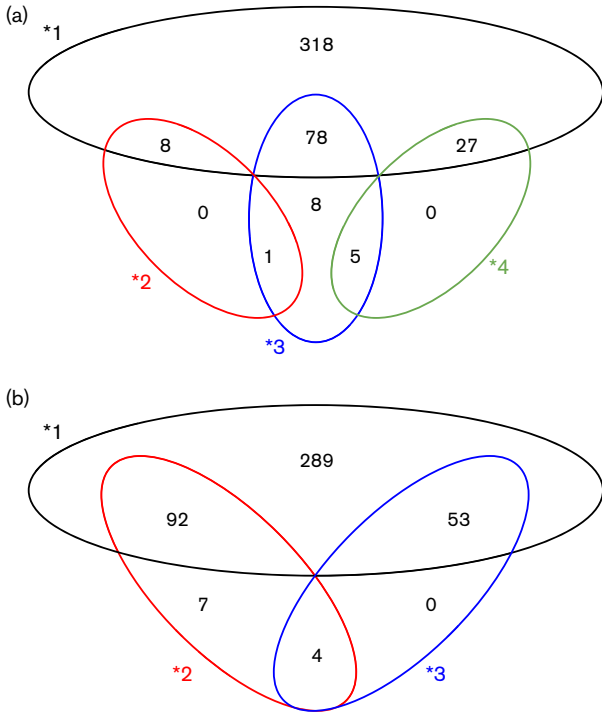


Distribution of (a) *CYP2C8* and (b) *CYP2C9* haplotypes in Coriell Personalized Medicine Collaborative participants in this analysis. For example, for *CYP2C8*, there were 318 *1/*1, 78 *1/*3, and 8 *3/*3 individuals.

### Table 1 Influence of *CYP2C8* and *CYP2C9* reduced metabolism haplotypes on ibuprofen dose

| Status | n | Ibuprofen users (n) | Reported typical ibuprofen dose [n (%)] | | |
|---|---|---|---|---|---|
| | | | Less* | Recommended | More |
| *1/*1 *CYP2C8* and *CYP2C9* | 254 | 204 | 9 (4.4) | 131 (64.2) | 47 (23.0) |
| Reduced metabolism *CYP2C8* or *CYP2C9* | 99 | 78 | 2 (2.6) | 44 (56.4) | 30 (38.5) |
| Reduced metabolism *CYP2C8* and *CYP2C9* | 92 | 69 | 10 (14.5) | 43 (62.3) | 14 (20.2) |

For *CYP2C8*, reduced metabolism indicates heterozygous, compound heterozygous, or homozygous for *2, *3, or *4 haplotypes. For *CYP2C9*, reduced metabolism indicates heterozygous, compound heterozygous, or homozygous for *2 or *3 haplotypes.
The number of participants who reported less than, recommended, and more than recommended typical doses of ibuprofen does not add up to the total number of participants who reported taking ibuprofen (and the corresponding percentages do not add up to 100%), because some participants who reported taking ibuprofen answered 'not applicable,' which they were instructed to do if they took the medication annually or less frequently.
Recommended over-the-counter dosage for ibuprofen is 200–400 mg every 4–6 h, not to exceed 1200 mg daily unless directed by a physician.
*P = 0.0171 (compared with a Holm–Bonferroni adjusted threshold of 0.025) for the effect of the number of reduced metabolism *CYP* haplotypes (possible values 0, 1, or 2) on the likelihood of a less than recommended reported typical ibuprofen dose.

### Table 2 Influence of combined *CYP2C8* and *CYP2C9* haplotypes on ibuprofen dose

| CYP2C8/ CYP2C9 status | n | Ibuprofen users (n) | Reported typical ibuprofen dose [n (%)] | | |
|---|---|---|---|---|---|
| | | | Less | Recommended | More |
| Ref/Ref | 254 | 204 | 9 | 131 | 47 |
| Het/Het | 77 | 57 | 8* | 36 | 12 |
| Ref/Het | 61 | 47 | 1 | 26 | 20 |
| Het/Ref | 33 | 26 | 1 | 14 | 9 |
| Hom/Het | 7 | 5 | 1 | 3 | 0 |
| Hom/Hom | 5 | 4 | 0 | 2 | 2 |
| Ref/Hom | 3 | 3 | 0 | 3 | 0 |
| Het/Hom | 3 | 3 | 1 | 2 | 0 |
| Hom/Ref | 2 | 2 | 0 | 1 | 1 |

Recommended over-the-counter dosage for ibuprofen is 200–400 mg every 4–6 h, not to exceed 1200 mg daily unless directed by a physician.
Het/Hom, heterozygous/homozygous (including compound heterozygous) for *CYP2C8* *2,*3, or *4 and *CYP2C9* *2 or *3 haplotypes; Ref,*1 haplotype for both *CYP2C8* and *CYP2C9*.
*P = 0.0215, compared with *1/*1.

### Table 3 Influence of *CYP2C8* and *CYP2C9* haplotypes on ibuprofen dose

| CYP2C8 | CYP2C9 | n | Ibuprofen users (n) | Reported typical ibuprofen dose [n (%)] | | |
|---|---|---|---|---|---|---|
| | | | | Less | Recommended | More |
| *1/*1 | *1/*1 | 254 | 204 | 9 | 131 | 47 |
| *1/*3 | *1/*2 | 67 | 50 | 8** | 33 | 8 |
| *1/*1 | *1/*3 | 45 | 33 | 1 | 18 | 13 |
| *1/*4 | *1/*1 | 21 | 16 | 1 | 8 | 7 |
| *1/*1 | *1/*2 | 16 | 14 | 0 | 8 | 7 |
| *1/*2 | *1/*1 | 7 | 5 | 0 | 4 | 1 |
| *1/*3 | *1/*1 | 5 | 5 | 0 | 2 | 1 |
| *3/*3 | *2/*2 | 4 | 3 | 0 | 2 | 1 |
| *1/*4 | *1/*3 | 4 | 1 | 0 | 1 | 0 |
| *3/*4 | *1/*2 | 4 | 2 | 1 | 1 | 0 |
| *1/*3 | *1/*3 | 3 | 3 | 0 | 0 | 3 |
| *3/*3 | *1/*2 | 2 | 2 | 0 | 1 | 0 |
| *1/*1 | *2/*2 | 2 | 2 | 0 | 2 | 0 |
| *1/*3 | *2/*3 | 2 | 2 | 1 | 1 | 0 |
| *1/*4 | *1/*2 | 2 | 2 | 0 | 1 | 1 |
| *2/*3 | *1/*2 | 1 | 1 | 0 | 1 | 0 |
| *1/*2 | *1/*3 | 1 | 1 | 0 | 1 | 0 |
| *3/*3 | *1/*1 | 1 | 1 | 0 | 0 | 1 |
| *3/*3 | *2/*3 | 1 | 1 | 0 | 0 | 1 |
| *3/*4 | *1/*1 | 1 | 1 | 0 | 1 | 0 |
| *1/*1 | *2/*3 | 1 | 1 | 0 | 1 | 0 |
| *1/*3 | *2/*2 | 1 | 1 | 0 | 1 | 0 |

Recommended over-the-counter dosage for ibuprofen is 200–400 mg every 4–6 h, not to exceed 1200 mg daily unless directed by a physician.
**P = 0.00872, compared with *1/*1.

slightly reduced (P = 0.0258, slightly greater than the Holm–Bonferroni adjusted threshold of 0.025), suggesting that reduced metabolism of both CYP2C8 and CYP2C9, rather than the combined number of reduced metabolism haplotypes, may be important for this association.

Tables 2 and 3 show similar information on the relationship between haplotype and ibuprofen dosing with haplotypes characterized more specifically: Table 2

stratifies by *CYP2C8*/*CYP2C9* reference, heterozygous or homozygous status, whereas Table 3 includes every haplotype combination observed in the study. In both cases, individuals with Het/Het *CYP2C8*/*CYP2C9* status (Table 2, $P = 0.0215$) and the *CYP2C8 \*1/\*3*, *CYP2C9 \*1/\*2* combined diplotype (Table 3, $P = 0.00872$) were significantly more likely than individuals with unaltered *CYP2C8* and *CYP2C9* to report taking less than the recommended dose of ibuprofen, and, in each case, individuals with altered metabolism status or diplotype were three to four times more likely to take a lower dose. Individuals with the linked *CYP2C8 \*1/\*3*, *CYP2C9 \*1/\*2* diplotype comprised 50 of 69 ibuprofen users with reduced metabolism haplotypes for both *CYP2C8* and *CYP2C9*, indicating that this combined, common diplotype is important for the observed association with ibuprofen dose adjustment.

Tables 4–6 present the side-effect information for each grouping of *CYP2C8* and *CYP2C9* status and haplotype. There do not seem to be meaningful relationships between haplotype and frequency of side effects, as reported by participants in this study, regardless of how

**Table 4**  Influence of *CYP2C8* and *CYP2C9* reduced metabolism haplotypes on ibuprofen side effects

| Status | n | Ibuprofen users (n) | Reported ibuprofen side effects (n) | | |
|---|---|---|---|---|---|
| | | | Abdominal or GI | Any | Count |
| *1/*1 *CYP2C8* and *CYP2C9* | 254 | 204 | 32 | 37 | 60 |
| Reduced metabolism *CYP2C8* or *CYP2C9* | 99 | 78 | 13 | 19 | 22 |
| Reduced metabolism *CYP2C8* and *CYP2C9* | 92 | 69 | 13 | 14 | 27 |

For *CYP2C8*, reduced metabolism indicates heterozygous, compound heterozygous, or homozygous for *2, *3, or *4 haplotypes. For *CYP2C9*, reduced metabolism indicates heterozygous, compound heterozygous, or homozygous for *2 or *3 haplotypes.
GI, gastrointestinal.

**Table 5**  Influence of combined *CYP2C8* and *CYP2C9* haplotypes on ibuprofen side effects

| CYP2C8/ CYP2C9 status | n | Ibuprofen users (n) | Reported ibuprofen side effects (n) | | |
|---|---|---|---|---|---|
| | | | Abdominal or GI | Any | Count |
| Ref/Ref | 254 | 204 | 32 | 37 | 60 |
| Het/Het | 77 | 57 | 10 | 11 | 24 |
| Ref/Het | 61 | 47 | 7 | 11 | 14 |
| Het/Ref | 33 | 26 | 4 | 6 | 6 |
| Hom/Het | 7 | 5 | 1 | 1 | 1 |
| Hom/Hom | 5 | 4 | 2 | 2 | 2 |
| Ref/Hom | 3 | 3 | 1 | 1 | 1 |
| Het/Hom | 3 | 3 | 0 | 0 | 0 |
| Hom/Ref | 2 | 2 | 1 | 1 | 1 |

GI, gastrointestinal; Het/Hom, heterozygous/homozygous (including compound heterozygous) for *CYP2C8* *2, *3, or *4 and *CYP2C9* *2 or *3 haplotypes; Ref, *1 haplotype for both *CYP2C8* and *CYP2C9*.

**Table 6**  Influence of *CYP2C8* and *CYP2C9* haplotypes on ibuprofen side effects

| CYP2C8 | CYP2C9 | n | Ibuprofen users (n) | Reported ibuprofen side effects (n) | | |
|---|---|---|---|---|---|---|
| | | | | Abdominal or GI | Any | Count |
| *1/*1 | *1/*1 | 254 | 204 | 32 | 37 | 60 |
| *1/*3 | *1/*2 | 67 | 50 | 8 | 9 | 22 |
| *1/*1 | *1/*3 | 45 | 33 | 6 | 8 | 11 |
| *1/*4 | *1/*1 | 21 | 16 | 3 | 5 | 5 |
| *1/*1 | *1/*2 | 16 | 14 | 1 | 3 | 3 |
| *1/*2 | *1/*1 | 7 | 5 | 1 | 1 | 1 |
| *1/*3 | *1/*1 | 5 | 5 | 0 | 0 | 0 |
| *3/*3 | *2/*2 | 4 | 3 | 1 | 1 | 1 |
| *1/*4 | *1/*3 | 4 | 1 | 1 | 1 | 1 |
| *3/*4 | *1/*2 | 4 | 2 | 0 | 0 | 0 |
| *1/*3 | *1/*3 | 3 | 3 | 1 | 1 | 1 |
| *3/*3 | *1/*2 | 2 | 2 | 0 | 0 | 0 |
| *1/*1 | *2/*2 | 2 | 2 | 0 | 0 | 0 |
| *1/*3 | *2/*3 | 2 | 2 | 0 | 0 | 0 |
| *1/*4 | *1/*2 | 2 | 2 | 0 | 0 | 0 |
| *2/*3 | *1/*2 | 1 | 1 | 1 | 1 | 1 |
| *1/*2 | *1/*3 | 1 | 1 | 0 | 0 | 0 |
| *3/*3 | *1/*1 | 1 | 1 | 1 | 1 | 1 |
| *3/*3 | *2/*3 | 1 | 1 | 1 | 1 | 1 |
| *3/*4 | *1/*1 | 1 | 1 | 0 | 0 | 0 |
| *1/*1 | *2/*3 | 1 | 1 | 1 | 1 | 1 |
| *1/*3 | *2/*2 | 1 | 1 | 0 | 0 | 0 |

'Abdominal or GI' includes individuals who reported either abdominal pain or GI-related side effects. 'Any' includes individuals who reported having at least one side effect of any kind. 'Count' is the total number of side effects reported to have occurred in each group.
GI, gastrointestinal.

side effects were evaluated (abdominal and gastrointestinal only, any side effect, or the total count of side effects). Ibuprofen is the most common NSAID involved in hypersensitivity reactions [13], but only three study participants reported 'Allergic reactions (hives, swelling, asthma, rash, itching)' as side effects from taking ibuprofen. Not surprisingly, these three individuals reported taking ibuprofen infrequently (never, annually, or monthly), and two of these three reported several other side effects when taking ibuprofen. The *CYP2C8* and *CYP2C9* diplotypes for these individuals were *1/*3, *1/*2; *1/*1, *1/*2; and *1/*1, *1/*2, but this sample size of three is too small to make any inferences about the relationship between *CYP2C8* and *CYP2C9* haplotypes and hypersensitivity reactions to ibuprofen.

## Discussion
We report that individuals in the CPMC with *CYP2C8* and *CYP2C9* reduced metabolism haplotypes are significantly more likely to take less than the recommended dose of ibuprofen for everyday pain. This suggests that some individuals with reduced ibuprofen metabolism may either recognize that they obtain adequate drug efficacy with lower doses or take lower doses because of previous side effects. Interestingly, though some individuals with reduced ibuprofen metabolism reduce their ibuprofen dose, most such individuals nevertheless take recommended or higher doses, potentially putting them at risk for side effects associated with ibuprofen and

other NSAIDs, such as gastrointestinal bleeding [21–23] or acute kidney injury [52].

The effects of *CYP2C8* and *CYP2C9* haplotype variants on ibuprofen metabolism have been fairly well characterized. For *CYP2C8*, the *2 haplotype, presents mainly in individuals of African [18] but not European [53] ancestry, does not seem to significantly alter either (*S*)-ibuprofen or (*R*)-ibuprofen metabolism *in vitro* [54], but has been shown to reduce metabolism of paclitaxel and arachidonic acid [53], and to alter metabolism of pioglitazone *in vitro* [55], indicating some effect on enzyme activity. Both the *CYP2C8* *3 and *4 haplotypes exhibit reduced clearance of both (*S*)-ibuprofen and (*R*)-ibuprofen *in vitro* [54], and studies in humans demonstrate similar reductions in ibuprofen clearance for *3 [35]. Although not as well-studied, the *4 haplotype also seems to alter CYP2C8 activity; for example, metabolic activity for paclitaxel was lower than the wild-type enzyme in human liver microsomes [56], and diclofenac 5-hydroxylation was reportedly altered in *4 carriers [57]. Taking these previous findings into consideration, each of the major *CYP2C8* haplotypes, *2, *3, and *4, were determined in this study and considered to be reduced metabolism haplotypes.

Previous studies of CYP2C9, the enzyme thought to be primarily responsible for ibuprofen metabolism [19,34, 58], showed that carriers of *2 and *3 haplotypes had lower (*S*)-ibuprofen clearance and higher (*S*)-ibuprofen area under the curve and half-life, and that *CYP2C9* carriers [15] had decreased (*R*)-ibuprofen clearance. Both the *2 and *3 haplotypes were determined in this study and considered to be reduced metabolism haplotypes. Notably, the *CYP2C8*3 allele is in partial linkage disequilibrium with the *CYP2C9*2 allele, which is associated with reduced metabolism of ibuprofen and other CYP2C9 substrates, and which occurs frequently only in European ancestry populations. In a study of the linkage between these two-star alleles in Swedish participants, 96% of the participants with *CYP2C8*3 also carried *CYP2C9*2, and 85% of the participants with *CYP2C9*2 also carried *CYP2C8*3 [59].

There was no observed relationship between *CYP2C8* and *CYP2C9* haplotypes or status and reported side effects. This could be because of several reasons. First, because some individuals with decreased ibuprofen metabolism were already taking lower than standard doses, they may have been avoiding side effects as a result. Second, side effects may have been occurring but were not apparent to participants; for example, one of the more serious side effects of ibuprofen (and other NSAID) overdose is acute kidney injury, which can have no clear signs or symptoms until severe loss of kidney function has occurred [52,60]. Third, it could be possible that the higher ibuprofen concentrations expected in individuals with reduced metabolism are not sufficiently high to

result in side effects, although there is evidence to the contrary in terms of both acute gastrointestinal bleeding [21] and emergency room visits as a result of NSAID overdose in sickle cell disease patients [36]. Fourth, because adverse events are rare, this study was likely underpowered to be able to detect a statistically significant relationship between metabolic status and side-effect frequency. A larger prospective or randomized controlled study would be needed to assess this relationship robustly.

The limitations of this study include, first, that ibuprofen pharmacokinetics are not known for these individuals, but inferred from *CYP2C8* and *CYP2C9* haplotypes. Ibuprofen pharmacokinetics also vary among individuals for nonpharmacogenetic reasons, including age, sex, health status, and other factors [61]. Second, ibuprofen usage information collected in this study was based on participant self-reported answers to questionnaires and may be subject to errors in reporting. Nonetheless, as over-the-counter pain medication use is not commonly tracked in electronic health records, self-report is likely the most practical way to collect this information. Third, the side-effect information collected focused on acute issues such as gastrointestinal upset or fatigue, and may not have been able to capture chronic conditions or those that are difficult to detect, such as acute kidney injury. Fourth, the participants included in this study were comparatively well educated: 72.8% had at least a bachelor's degree, compared with ~33% of the US population. As a result, they may have felt more confident in making decisions related to their medical care and were more likely to be receiving regular, higher quality medical care. It is not known how results from this analysis may be extrapolated to broader US or global populations.

Pharmacogenomic screening for *CYP2C8* and *CYP2C9* haplotypes has the potential to reduce morbidity and mortality associated with NSAID use. Up to 20% of the US population have *CYP2C8* and *CYP2C9* diplotypes associated with ibuprofen exposures increased 50% or greater above typical [35,62,63]. Information about the potential for higher exposures of ibuprofen, if presented to individuals or their doctors, could result in behavior modification [41,42] such as dose adjustment or selection of alternative medication, potentially reducing morbidity rates. Although establishing that individuals with reduced ibuprofen metabolism are at an elevated risk for NSAID-related side effects would require larger cohorts and long-term study, it is clear, at present, that such individuals are likely experiencing higher than anticipated ibuprofen concentrations, to the extent that some are able to accurately judge that they should take lower doses. Additional research on variants that influence ibuprofen metabolism is necessary to better understand the relationships between haplotype, pharmacokinetics, pain relief efficacy, and side-effect risk.

## Conflicts of interest
There are no conflicts of interest.

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