Web-based phenotyping yields replication of genetic associations with sensitivity to warfarin and side effects from ibuprofen

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Goals of study

Progress in pharmacogenomics research has been hampered by the cost and time required to bring together, genotype, and characterize sufficiently large cohorts of individuals who use various medications. The primary goal of this study was to assess how well 23andMe's highly scalable, web-based research platform performs in terms of discovering genetic associations with drug efficacy and toxicity. Here we focus on three classes of drugs – warfarin, non-steroidal anti-inflammatory drugs (NSAIDs), and proton pump inhibitors (PPIs) – for which some pharmacogenomic information is available. In the first phase of this study we assessed how well web-based questionnaires elucidate drug response data by comparing online self-reported data to responses obtained via semi-structured telephone interviews. The aim of the second phase of this project, the focus of this poster, was to determine whether we could replicate known associations between warfarin sensitivity and variants in the *CYP2C9* & *VKORC1* genes, between reactions to NSAIDs and variants in the *CYP2C9* & *CYP2C8* genes, and between reactions to PPIs and variants in the *CYP2C19* gene.

Methods

Customers who gave prior indication of use of warfarin, NSAIDs, or PPIs completed online surveys on their use of and response to the relevant medication. All participants had been genotyped on 23andMe's custom version of an Illumina array with between 500,000 and 1 million single nucleotide polymorphisms (SNPs). All data included in this study were from customers who had consented to research participation.

Warfarin

Over 1000 individuals completed a warfarin survey. We predicted warfarin sensitivity based on genotypes for SNPs in the *CYP2C9* (rs1057910, rs1799853) and *VKORC1* (rs9923231) genes (1,2,3). We assigned each respondent a "sensitivity" score (low, intermediate, high) based on their survey responses related to dosage, change in dosage, bleeding and blood clots. Regression analyses were conducted in a set of respondents with European ancestry, controlling for sex, age, population structure, and body mass index (BMI).

NSAIDs

Over 20,000 individuals completed an NSAIDs survey. We predicted response to NSAIDs (ibuprofen, naproxen, aspirin) based on genotypes for SNPs in the *CYP2C9* (rs1057910, rs1799853) and *CYP2C8* (rs11572080, rs10509681) genes (4,5,6). We took two approaches:

1) Testing for association with each of the 4 individual SNPs
2) Testing for association with a metabolization score based on two SNPs in the *CYP2C9* gene.
   - Typical metabolizer: *1/*1
   - Intermediate: *1/*2 or *1/*3
   - Poor: *2/*2 or *2/*3 or *3/*3

We considered two sets of cases based on survey responses: (1) "any serious gastrointestinal problem" & (2) "any serious gastrointestinal problem" or "stomach pain". Regression analyses were conducted in a set of respondents with European ancestry, controlling for sex, age, and population structure.

PPIs

Over 2000 individuals completed a PPIs survey. We predicted PPI metabolism (ultra, extensive, intermediate or poor) given genotypes for SNPs in the *CYP2C19* gene (rs4244285, rs28399504, rs12248560) (7,8). We considered two sets of PPIs:

1) All in survey [omeprazole, omeprazole - rapid release, lansoprazole, dexlansoprazole, esomeprazole, pantoprazole, rabeprazole, other]
2) Four PPIs understood to act primarily through *CYP2C19* (rabeprazole, omeprazole, omeprazole - rapid release, dexlansoprazole)

We considered both reported efficacy and reported side effects:

- We assigned each respondent an overall "efficacy" score from 1 to 5 based on their responses to the PPIs survey
- We considered three sets of individuals to be cases:
  - Those who reported any side effect
  - Those who reported any gastrointestinal problems
  - Those who reported headaches or dizziness

Regression analyses were conducted in a set of respondents with European ancestry, controlling for sex, age, population structure, and BMI.

Results

Phase 1: Summary of results

Web-based surveys performed well in terms of obtaining medication response and side effects information. Agreement between web- and phone-based surveys was good (>70% for most questions). Reporting for prescription medications taken currently proved more reliable than for medications taken in the past and for medications taken as needed.

Phase 2: Warfarin

We examined correspondence between warfarin sensitivity based on self report with that predicted from *CYP2C9* and *VKORC1* genotypes (Table 1).

Table 1: Warfarin: Counts (and corresponding percentage) of individuals in each genetic and self-report category of sensitivity to warfarin.

self-report / genetic	low	intermediate	high
lower than avg	84 (5.5%)	240 (15.7%)	57 (3.7%)
average	106 (6.9%)	364 (23.8%)	106 (6.9%)
higher than avg	87 (5.7%)	360 (23.5%)	121 (7.9%)
much higher than avg	2 (0.1%)	1 (0.1%)	3 (0.2%)

Association between genetic and self-reported sensitivity is highly significant: p = 1.4e-13. BMI also plays an important role: p = 0.0057.

Phase 2: NSAIDs

We first looked for associations between gastrointestinal problems (GI) in response to all NSAIDs or individual NSAIDs and each of the SNPs (Table 2).

Table 2. NSAIDs: Analysis of association between "any serious gastrointestinal problems or stomach pain" (GI problems) and *CYP2C8* and *CYP2C9* variants. p-values < 0.05 are indicated in bold.

Variant	*CYP2C9*2*	*CYP2C9*3*	*CYP2C8*3*	*CYP2C8*3*
SNP RSID	rs1799853	rs1057910	rs11572080	rs10509681
any NSAID	0.060	0.70	**0.019**	**0.032**
aspirin	0.12	0.88	0.061	0.065
ibuprofen	**0.0059**	0.35	**0.027**	**0.045**
naproxen	**0.020**	0.52	0.22	0.11

For aspirin, not known to be metabolized through *CYP2C9* or *CYP2C8* (9), we do not see association between GI problems and these variants. Associations were strongest for Ibuprofen. We therefore examined more closely the association between GI side effects and *CYP2C9* genotypes for ibuprofen.

Association between ibuprofen-related GI problems and *CYP2C9* genotypes was strongest for comparison of poor and typical metabolizers (predicted by *CYP2C9* genotypes), with an odds ratio of 1.4 (Table 3).

Table 3. NSAIDs: Analysis of association between "any serious gastrointestinal problems or stomach pain" and metabolizer type (typical, intermediate, poor) predicted from *CYP2C9* genotype.

	p-value	effect (odds ratio)
intermediate vs typical	0.054	1.12 (1.00-1.26)
poor vs typical	0.0086	1.40 (1.09-1.79)

Phase 2: PPIs

We tested for association between response to PPIs and *CYP2C19* genotypes (Table 4).

Table 4. Tests for association between *CYP2C19* genotypes and PPI response; 2-sided p-values, controlling for sex and age, for European participants who had completed PPIs survey and were taking or had taken a PPI in the past.

	efficacy score	any side effect	gi problems	headaches or dizziness
all PPIs, no BMI	0.82	0.23	0.79	0.56
all PPIs, BMI	0.76	0.24	0.69	0.52
PPIs subset, no BMI	0.93	0.34	0.61	0.82
PPIs subset, BMI	0.93	0.32	0.80	0.71

None of the responses to PPIs appear to be associated with *CYP2C19* genotypes.

Conclusions & Discussion

We successfully replicated the more established associations:

- Warfarin sensitivity/*CYP2C9* & *VKORC1* association replicated strongly
- Ibuprofen side effects are associated with *CYP2C9*; association did not extend to all NSAIDs

We did not detect any association between response to PPIs (efficacy or side effects) and *CYP2C19* SNPs or genotypes, despite examining multiple outcomes. Possible explanations include:

- Sample sizes are relatively small so power is insufficient
- At typical PPI dosages, few participants experience low efficacy & side effects are rare, so there is no association to be detected

This study indicates that 23andMe's innovative research platform is powered to discover associations between genetic variants and drug response (efficacy or side effects). Results reflect the drug responses that users notice and can report, rather than pharmacokinetics.

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