Measures of Exposure Impact Genetic Association Studies: An Example in Vitamin K Levels and *VKORC1*

Dana C. Crawford[1], Kristin Brown-Gentry[2], Mark J. Rieder[3]

[1]Institute for Computational Biology, Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH 44106, USA
[2]Center for Human Genetics Research, Vanderbilt University, Nashville, TN 37232, USA
[3]Adaptive Biotechnologies Corporation, Seattle, WA 98102, USA

INTRODUCTION

- Gene-environment (GxE) studies for common human diseases have been relatively few, in part because the environment or exposure is difficult to measure.
- Most large-scale epidemiologic studies rely on questionnaires to assess and measure past and current exposure levels for GxE studies. Questionnaires are cost-effective; however, the data may or may not accurately represent the exposure compared with direct measurements.
- Much like phenotyping, the choice in how an exposure is measured may impact downstream tests of genetic association and GxE interaction studies.
- As a case study, we performed tests of association between five common *VKORC1* SNPs and two different measurements of vitamin K levels, dietary (n=5,725) and serum (n=348), in the Third National Health and Nutrition Examination Surveys (NHANES III). We compared results from these tests of association.

STUDY POPULATION

- NHANES is conducted by the Centers for Disease Control and Prevention (CDC) and ascertains non-institutionalized US participants regardless of health status at the time of survey. NHANES collects health and lifestyle survey data, laboratory data, and exam data.
- All physical examinations are performed in the Mobile Examination Center (MEC) unless the participant is physically unable.
- DNA was collected for Phase 2 of NHANES III between 1991 and 1994 (n=7,159)

Vitamin K levels were measured in two ways in NHANES III (Table 1):

- Dietary vitamin K levels were measured in NHANES III as total nutrient intake of vitamin K (mcg) collected from the 24-hour dietary recall performed in the MEC. These data were collected in collaboration between CDC and the University of Minnesota's Nutrition Coordinating Center.
- Serum vitamin K (phylloquinone; ng/ml) was measured using reverse phase HPLC in non-Hispanic white women ages 6-29 years in Phase 2 of NHANES III. The lower limit of detection was 0.05, and the range of serum vitamin K levels in the subset of NHANES samples tested was 0.05ng/ml – 6.799ng/ml.

Table 1. Study population characteristics. Unweighted descriptive statistics are shown for basic demographic variables (sex, age, and body mass index) as well as the two measures of vitamin K levels. Sample sizes shown are for participants with dietary vitamin K levels available. Serum vitamin K levels were only measured in non-Hispanic white women ages 6-29 years in Phase 2 of NHANES III (n=348). Abbreviations: standard deviation (SD), natural log (ln).

	Non-Hispanic whites	Non-Hispanic blacks	Mexican Americans
Sample size	2,344	1,675	1,706
% female	61	58	50
Mean (± SD) age in years	53.46 (20.32)	40.79 (16.71)	41.15 (17.42)
Mean (±SD) body mass index (kg/m^2)	26.66 (5.6)	27.3 (369.6)	27.1 (422.6)
Mean (±SD) ln(dietary vitamin K) (mcg)	4.05 (0.97)	4.02 (1.29)	3.79 (0.99)
Mean (±SD) ln(serum vitamin K) (mg/dl)	-1.30 (0.79)	-	-

METHODS

- We genotyped five *VKORC1* tagSNPs using Sequenom and TaqMan: rs2884737, rs9923231, rs9934438, rs8050894, and rs2359612.
- Single SNP tests of association were performed unadjusted and adjusted using linear regression assuming an additive genetic model stratified by race/ethnicity. Two dependent variables were tested: dietary vitamin K levels and serum vitamin K levels, both log transformed.
- All analyses were conducted remotely in SAS v9.2 and SUDAAN using the Analytic Data Research by Email (ANDRE) portal of the CDC Research Data Center in Hyattsville, MD.

RESULTS

Figure 1. Results of tests of association between *VKORC1* common variation and dietary levels of vitamin K levels stratified by race/ethnicity. Tests of association were performed using linear regression assuming an additive genetic model and adjusted for sex, age, body mass index, current smoking status, dietary calcium, phosphorous, magnesium, iron, zinc, copper, sodium, potassium, protein, carbohydrates, fiber, total vitamin A, total carotenes, total alpha-tocopherol equivalents, vitamin C, vitamin B6, vitamin B12, folic acid, and total calories. Results (p-values and betas) are plotted by rs number and race/ethnicity (coded by color) using Synthesis View. The direction of the triangles represents the direction of the genetic effect. The red line represents a p-value threshold of 0.05.

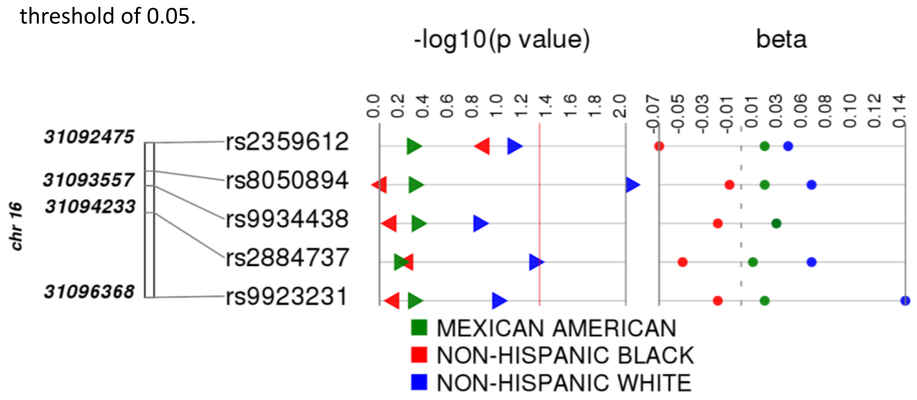
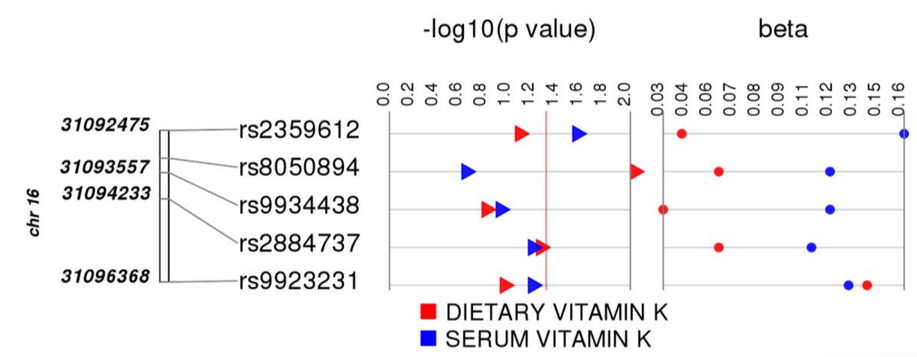


Figure 2. Comparison of results of tests of association between *VKORC1* common variation and vitamin K levels by vitamin K measurement. Tests of association were performed using linear regression assuming an additive genetic model and adjusted for sex (for model with dietary vitamin K levels) and the other variables listed in Figure 1. Results (p-values and betas) are plotted by rs number and vitamin K measurement (coded by color) using Synthesis View. The direction of the triangles represents the direction of the genetic effect. The red line represents a p-value threshold of 0.05.



CONCLUSIONS

- We did not replicate previously reported associations, and observed associations were dependent on vitamin K measurement.
- Although the sample size impacted power, this is a cautionary example for GxE studies.