MENDELIAN RANDOMIZATION TO ASSESS THE CAUSAL ROLE OF DIETARY CALCIUM ON COLORECTAL CANCER RISK
Background

- Some observational studies have reported an inverse relationship between dietary calcium intake and risk of colorectal cancer (CRC).
  - A pooled analysis of 10 cohort studies, which assessed dietary calcium and total calcium intake (diet plus supplements), found a reduction in the incidence of colorectal cancer (Cho et al. 2004).

- However, other studies have reported no association.
  - The only clinical trial to examine this association found daily supplementation of calcium (with vitamin D) for seven years had no effect on the incidence of CRC (Wactawski et al. 2006).
Background

- Unmeasured or unaccounted confounding in observational studies could contribute to these mixed results.
- Mendelian randomization (MR) could overcome this issue.
Background

- A MR study uses genetic variant/s randomly allocated at conception as an instrumental variable for the exposure of interest.

- This ad-hoc approach is less vulnerable to confounding than observational studies, and is more cost-effective than performing a clinical trial.
Objective

Use MR to reassess the role of dietary calcium intake on CRC risk
Study population

Participants included 10,527 individuals with confirmed CRC and 10,900 population-based controls from the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO) and the Colon Cancer Family Registry (CCFR).
Mendelian randomization: genetic variant as an instrumental variable

G → X → Y

Modifiable risk factor:

C

Confounders:

Outcome:
Mendelian randomization: genetic variant as an instrumental variable

Variant rs4988235 in *MCM6* was used as an instrumental variable for dietary calcium; this marker is used in genetic tests of lactose intolerance.

Note: The primary contributor of dietary calcium in the United States is dairy intake (specifically milk).
Mendelian randomization: genetic variant as an instrumental variable

Instrumental genetic variable: rs4988235

Modifiable risk factor: Dietary calcium

Outcome: Colorectal cancer

Confounders: “Healthy” dietary/lifestyle patterns
Mendelian randomization approach

- We used a 2-stage MR approach to estimate the causal association of dietary calcium intake with CRC risk.
  - 1) Fit a multivariable linear regression model to predict dietary calcium.
  - 2) Using a multivariable logistic regression model, examine the association between genetically predicted dietary calcium and CRC risk.
- In each stage we adjusted for age, sex, BMI, study, and the first 3 principal components of genetic ancestry.
## Results

**Table 1: Characteristics of study population by rs4988235 genotype**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>Overall</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of participants</td>
<td>15%</td>
<td>41%</td>
<td>44%</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>64.5 (9.5)</td>
<td>64.8 (9.4)</td>
<td>64.7 (9.0)</td>
<td>64.7 (9.3)</td>
<td>0.42</td>
</tr>
<tr>
<td>Women, %</td>
<td>51%</td>
<td>55%</td>
<td>57%</td>
<td>55%</td>
<td>1.03E-05</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.5 (4.4)</td>
<td>26.6 (4.6)</td>
<td>26.8 (4.7)</td>
<td>26.7 (4.6)</td>
<td>9.21E-03</td>
</tr>
<tr>
<td>Dietary calcium, mg/day</td>
<td>697.1 (461.2)</td>
<td>780.8 (512.8)</td>
<td>830.6 (529.5)</td>
<td>790.6 (515.0)</td>
<td>&lt;2E-16</td>
</tr>
<tr>
<td>Total calcium, mg/day</td>
<td>834.9 (630.1)</td>
<td>927.9 (682.7)</td>
<td>986.1 (683.5)</td>
<td>940.1 (677.5)</td>
<td>&lt;2E-16</td>
</tr>
<tr>
<td>Colorectal cancer, %</td>
<td>48%</td>
<td>46%</td>
<td>47%</td>
<td>47%</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Data are presented as either percentages or as means (SD)

χ² test for categorical variables and ANOVA test for linear associations according to the number of T alleles

*Of those with calcium data
Results

Table 2: Results of 1\textsuperscript{st} stage of Mendelian randomization

<table>
<thead>
<tr>
<th>SNP</th>
<th>Gene</th>
<th>N</th>
<th>CAF</th>
<th>Imputation quality</th>
<th>β</th>
<th>P</th>
<th>L95</th>
<th>U95</th>
<th>F stat</th>
<th>Adjusted R(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4988235</td>
<td>MCM6</td>
<td>16,503</td>
<td>0.65</td>
<td>0.91-1.00</td>
<td>61.71</td>
<td>1.98E-26</td>
<td>50.36</td>
<td>73.06</td>
<td>116.8</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Results from the multivariable linear regression (additive) model to predict dietary calcium (mg/day) on participants with dietary calcium data; adjusted for age, sex, BMI, study, and the first 3 principal components of genetic ancestry.
Table 3: Results of 2\textsuperscript{nd} stage of Mendelian randomization

<table>
<thead>
<tr>
<th>Predictor</th>
<th>OR</th>
<th>P</th>
<th>L95*</th>
<th>U95*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predicted dietary calcium (per 300 mg/dL)</td>
<td>0.95</td>
<td>0.63</td>
<td>0.77</td>
<td>1.13</td>
</tr>
</tbody>
</table>

Results from logistic regression on all participants; adjusted for age, sex, BMI, study, and the first 3 principal components of genetic ancestry

*SE calculated using the delta method
Our findings rely on the following assumptions

1. **G and X are robustly associated**

   In the 1st stage, rs4988235 was found to be robustly associated with dietary calcium (F statistic = 116.9); an F statistic > 10 is considered to be robust (Staiger et al. 1997).
Our findings rely on the following assumptions:

1. **G** and **X** are robustly associated
2. **G** is unrelated to **C**; no common causes of **G** and **Y**

Individuals’ genotypes are randomized at conception and thus not subject to non-genetic confounders and reverse causation.
Our findings rely on the following assumptions:

1. G and X are robustly associated
2. G is unrelated to C; no common causes of G and Y
3. G is related to Y only through its association with X

Variant rs4988235, directly influences the production of the lactase enzyme, and thus the digestion of lactose. Since lactose is only found in dairy products, this variant should only influence dairy consumption. Dairy intake serves as the largest contributor of dietary calcium in the U.S. (Subar et al. 1998).
Conclusions

- Did not detect a statistically significant effect between genetically predicted dietary calcium intake and CRC risk.
  - Our estimate, suggests a slight protective effect of dietary calcium on CRC risk.

- Despite our sizable study population and robust IV, even larger scale MR analyses may be required to corroborate our findings.