On the detection of pleiotropic QTLs in extended pedigree data: evaluation of different multi-trait association approaches

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Materials and Methods

Background

- Joint analysis of correlated phenotypes can provide greater power to map underlying quantitative loci (QTLs) with pleiotropic effects than univariate analysis of the individual phenotypes [1].
- Various methods to perform multivariate association tests in population- or family-based data have been proposed:
  - In GWAS of unrelated individuals, bivariate association analysis based on a Seemingly Unrelated Regression (SUR) model [2] provides, on average, greater power than univariate analysis [3].
  - In data from extended pedigrees, the estimation of the covariance structure makes it difficult to fit bivariate models. An intuitive approach to detect a QTL with pleiotropic effects is to fit a univariate model on the first principal component (PC1) obtained from principal component analysis (PCA) of the phenotypes of interest.

- Here, we compare different approaches to detect a QTL with pleiotropic effects using the example of two highly correlated cardiac phenotypes measured in an extended-pedigree study.

Table 3. Top 11 most associated loci from SUR-based bivariate GWAS: comparison with results from univariate association analyses of QTs, RRs, PC1, and QTs/RRs.

<table>
<thead>
<tr>
<th>SNP</th>
<th>1A/1A (Ref. All.)</th>
<th>MAF</th>
<th>SUR</th>
<th>Univariate analysis of QTs</th>
<th>Univariate analysis of RRs</th>
<th>Univariate analysis of PC1</th>
<th>Univariate analysis of QTs/RRs</th>
<th>Ranks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rs1</td>
<td>A/G (G)</td>
<td>0.30</td>
<td>2.4E-07</td>
<td>Mean (QTs) = 0.01</td>
<td>Mean (RRs) = 0.25</td>
<td>Mean (PC1) = 0.13</td>
<td>Mean (QTs/RRs) = 0.23</td>
<td>0.31</td>
</tr>
<tr>
<td>Rs2</td>
<td>A/G (G)</td>
<td>0.38</td>
<td>1.9E-07</td>
<td>Mean (QTs) = 0.01</td>
<td>Mean (RRs) = 0.19</td>
<td>Mean (PC1) = 0.19</td>
<td>Mean (QTs/RRs) = 0.39</td>
<td>0.50</td>
</tr>
<tr>
<td>Rs3</td>
<td>A/C (A)</td>
<td>0.19</td>
<td>9.7E-06</td>
<td>Mean (QTs) = 0.04</td>
<td>Mean (RRs) = 0.19</td>
<td>Mean (PC1) = 0.14</td>
<td>Mean (QTs/RRs) = 0.56</td>
<td>0.60</td>
</tr>
<tr>
<td>Rs4</td>
<td>T/C (T)</td>
<td>0.21</td>
<td>2.8E-06</td>
<td>Mean (QTs) = 0.06</td>
<td>Mean (RRs) = 0.20</td>
<td>Mean (PC1) = 0.13</td>
<td>Mean (QTs/RRs) = 0.41</td>
<td>0.60</td>
</tr>
<tr>
<td>Rs5 (rs2880058)</td>
<td>G/A (G)</td>
<td>0.38</td>
<td>1.8E-07</td>
<td>Mean (QTs) = 0.05</td>
<td>Mean (RRs) = 0.08</td>
<td>Mean (PC1) = 0.15</td>
<td>Mean (QTs/RRs) = 0.12</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Genome-wide significant SNPs after a Bonferroni correction are shown in bold. "A1" is the minor allele: "Minor allele frequency," "Unadjusted univariate P-values," "Ranks of the identified SNP"

Study sample.

In the framework of the MICROS study, that was carried out in 3 isolated villages in South Tyrol (Italian Alps) [4], we consider here 942 individuals with available electrocardiogram (ECG) data. All samples were genotyped with Illumina HumanHap300 SNP microarrays: 306,622 SNPs were available for analysis after quality control (SNP call rate ≥ 98%, individual call rate ≥ 98%, Hardy-Weinberg equilibrium test p-value > 1E-06, minor allele frequency ≥ 1%).

Phenotypes.

The QT and RR intervals, measured by the ECG. Individuals were excluded according to atrial fibrillation and QRS interval >120 ms. QT and RR were standardized before analysis.

Association analysis.

- Univariate GWAS on QTs, RRs, and PC1 were performed using the mmsgore function in GenABEL [5] and estimating the covariance matrix from the genomic kinship matrix. For biological interpretation we also fit a GWAS on QTs adjusted by RRs (QTs/RRs).

- Bivariate GWAS on QTs and RRs was performed using a SUR model with the Systemfit package, using previously described methods [3], and considering the samples as unrelated.

- All models were adjusted for age, sex, BMI and study location and in all models an additive genetic effect was assumed. The Bonferroni corrected threshold for statistical significance was 3.26x10^-5.

Results

We report the top 11 bivariate associated loci in Table 3. Given the lack of a method to account for the pedigree structure in the bivariate SUR analysis, we provide the ranks of the p-value statistics to allow a fair comparison between methods. In summary:

- Rs1, 2, and 3 were strongly associated also with QTs, RRs and PC1, but not with QTs/RRs. A similar behavior was observed for Rs4 but with a weaker effect on RRs.

- Simultaneous, strong association of bivariate and both univariate analyses (QTs and RRs) may suggest that an underlying QTL acts on both traits, reflecting a QTL with pleiotropic effects.

- Rs5 was strongly associated also with QTs, PC1, and QTs/RRs but not with RRs.

Discussion/Conclusions

Our bivariate genome-wide association scan using the SUR model appears to be a valuable and powerful screening approach to identify loci with a potential effect on both the traits involved in the analysis. In fact, four of top 11 loci identified had relevant association statistics also when the individual traits were analyzed separately. The analysis of the first principal component further supported these findings.

In other cases, the signals in the bivariate analysis were driven by one of the two traits, as it was the case for the rs5 located 25 kb upstream of NOS1AP, that has been previously identified for the QT interval [6].

Limitation of the SUR method is that the hypothesis testing framework allows for multiple alternatives, i.e.: the null hypothesis can be rejected when the locus is associated with at least one of the two traits. For this reason, the method needs to be coupled with univariate approaches to allow a correct interpretation. Methodological developments to include correction for individuals’ relatedness are also ground for further research.

References