Supplemental Data

Inference of the Genetic Architecture Underlying BMI and Height with the Use of 20,240 Sibling Pairs

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Figure S1. Volta plots showing recombination traces in QSIBs

Examples of IBD estimates (y-axis) for all positions (x-axis) at four chromosomes (vertical panels) for three QSIBs (horizontal panels) are shown. With SNP data the precision of IBD calculation is high. Thus, recombination events can be cleanly demarcated by sudden changes of IBD along the chromosome.
Figure S2. Comparison of heritability estimates using standard design and QISP design of genetic relationship matrices

The QISP design of the GRM comprises 2x2 block diagonal matrices, where each block diagonal represents a sib-pair. The standard design comprises block diagonals of size $s \times s$, where $s$ is the number of sibs in the nuclear family. Phenotypes with heritabilities of $h^2 = \{0, 0.25, 0.5, 0.75\}$ (x-axis) and a common environment component of $c^2 = 0.1$ were simulated for the Framingham cohort (4607 QISPs, 4355 unique individuals), and heritability estimates were calculated (y-axis) using both the standard and QISP designs. This was repeated 50 times. It can be seen that the QISP design is equivalent to the standard design.
The variance components method, as implemented in the Merlin software package, performs linkage analysis using the variance components method. However, this is computationally slow for permutations and simulations. Here is shown the level of agreement of LOD scores from the linkage analysis as performed by Haseman-Elston regression (HE), the Visscher-Hopper method (VH), and the variance components method (VC). Upper panels are correlation coefficients.
Figure S4. Relationship between combined and meta-analyses

The method of using within-family genetic variation should guard against the possibility of inflation due to stratification, as demonstrated by the close agreement between the combined analysis (linkage on all 20240 sib-pairs) and the meta-analysis (post-hoc calculation of overall test statistics from accumulated results of linkage analysis from each independent cohort). The correlation for BMI (top) is $r = 0.97$ and for height (bottom) is $r = 0.95$. 
Figure S5. Example of effect distributions used in simulations

Rows correspond to (top) Null model – no genetic effects; (2nd row) C1 – One QTL per chromosome; (3rd row) C2 – Polygenic effect, effects evenly distributed across genome; (4th row) C4 – Polygenic effect, all SNPs have an effect but clusters are chosen to have much larger effects than background. R1 and R2 are rare models produced by sampling SNPs for each sib-pair’s total genetic variation, where the probability of a SNP being chosen is proportional to the effect size in C2 and C3 respectively.
Figure S6. Effect of extreme population stratification on genomic inflation factor for within-family linkage analysis

Phenotypes were simulated for 20,000 QISPs from five independent cohorts. Phenotypes had no genetic effects, but had either a very large cohort effect (right boxplot, variance explained by cohort effect = 20%), or no cohort effect (left boxplot). Linkage analysis was performed on these phenotypes and genomic inflation factors were calculated (y-axis). This was repeated 100 times for each scenario.
Phenotypes were simulated for five different cohorts such that each chromosome only had genetic effects contributing to a single cohort. Genetic effects across cohorts were then scaled such that each cohort had identical heritabilities of 0, 0.25, 0.5, 0.75, or 1 (x-axis). Heritability estimates were then calculated for the combined data (y-axis). This was performed 50 times for each simulated heritability value.
Figure S8. Estimation of genomic inflation using a within-family linkage analysis for phenotypes with extreme genetic heterogeneity

Phenotypes were simulated for five different cohorts such that each chromosome only had genetic effects contributing to a single cohort. Genetic effects across cohorts were then scaled such that each cohort had identical heritabilities of 0, 0.25, 0.5, 0.75, or 1 (point colours). Linkage analysis was then performed on each independent cohort, the combined cohort, and also a meta analysis was performed for all cohorts. This was performed 50 times for each simulated heritability value. The genomic inflation factor for each linkage analysis was calculated, and the mean values are plotted here (y-axis) against sample size (x-axis). The combined data and meta-analysed data have a sample size of 20,240 (far right of plot). It is evident that combining genetically heterogeneous samples does not lead to increased genomic inflation in within-family linkage analysis.
Figure S9. Distribution of genome wide IBD between QISPs

The distribution of genome wide IBD sharing for 20240 QSIBs is shown. The coefficients are normally distributed with mean of 0.500 and standard deviation of 0.037.
Age difference at time of measurement for BMI

Figure S10. Effect on difference of age at time of measurement on BMI correlations

Top left: Correlation coefficient for QISPs (y-axis) for groups of siblings who were measured at different ages, where the age of measurement difference is denoted on the x-axis. (i.e. 30 on the x-axis is all QISPs whose age of measurement difference is between 25 and 30). Top right: Number of sib pairs in each age-difference bracket. Bottom left: As in the top left, but showing the correlation coefficient as QISPs with larger age of measurement differences being added cumulatively. Bottom right: The change in the correlation for the sample as more QISPs with increasing age of measurement differences are added.
Figure S11. Relationship between genetic variance and chromosome size

Panels are divided vertically by trait (BMI and height), and horizontally by estimation method (C = REML estimates are constrained to be within the range of 0 and 1, NC = REML estimates are not constrained). Heritability estimates for each chromosome are plotted with standard-error bars, and blue lines represent least squares regression estimates of the relationship.
Figure S12. Q-Q plots from linkage analysis, using all markers

For each cohort (left five panels) as well as the combined dataset and the p-values from the meta-analysis (right two panels), and for both BMI (top) and height (bottom), Q-Q plots were produced to demonstrate overall genomic inflation as being a departure from the expectation (x=y line). 95% confidence intervals are shaded in grey, and GC values for each cell represent genomic inflation. All markers are used, so large correlations exist between tests, which violates the assumptions behind Q-Q plots. Figure 1 shows identical plots but using only pruned markers to avoid violating the assumption of independence.
Figure S13. Genomic inflation comparison between simulations and empirical results

The genomic inflation factor $\lambda_{GC} = \frac{\chi^2(1-p_m, 1)}{\chi^2(0.5, 1)}$ where $p_m$ is the median p-value from the linkage scan, is plotted for all simulations (box-whisker plots) and for corresponding actual linkage scans performed on original data (horizontal lines). The sample sizes are denoted in columns of panels, representing from left to right the TwinsUK study, Netherlands Twin Registry, TWINGENE study, Framingham Heart Study, QIMR, and the combined dataset. Simulated heritability values for the simulations correspond to BMI (top row) and height (bottom row). Box whisker plots represent the distribution of $\lambda_{GC}$ values for each replicate within the specific model / heritability / sample size simulation. Genetic models for the different simulations are labeled on the x-axis, according to the key in Figure 4. It can be shown that genomic inflation is expected under a polygenic model with sufficient power, and this is also observed in the original linkage analyses (Figure 2).
Figure S14. Example of Haseman-Elston regression for large QTL

The Haseman-Elston regressions for the highest LOD scores for height (left) and BMI (right) are shown here. After removing outliers $p_D = (y_1 - y_2)^2$ is plotted against the estimated IBD at the relevant position, for all 20,240 sib-pairs. A regression line (red) is plotted. For height, intercept $a = 1.45$ and regression coefficient $b = -0.16, p = 5.8 \times 10^{-7}$. For BMI, $a = 1.12, b = -0.20, p = 1.4^{-5}$. 
Figure S15. Permutation analysis of maximum LOD scores

For each cohort (5 bars to the left) and for the combined sample set (bar furthest to the right) an independent permutation analysis was performed such that the entire family of tests was rerun with QISP phenotypes randomly relabeled from QISP genomic IBD scores. Thus, each box-whisker plot represents the distribution of maximum LOD scores from the 500 permutations per cohort, with red points representing the maximum LOD score achieved in the true linkage scans. As sample size increases linkage power improves, shown by the constant distribution of LOD scores under the null but increasing LOD scores for the linkage analyses.
Figure S16. Threshold for linkage analysis with background polygenic effects

The maximum LOD scores from each polygenic simulation ($n = 2400$ for each heritability value) are plotted in order of magnitude, and the 95% largest value (indicated by vertical lines) is used to establish the threshold at experiment-wide $\alpha = 0.05$ (shown by horizontal lines). This demonstrates that empirical thresholds are determined by trait heritability.
Figure S17. Relationship between number of QTLs reported and chromosome size

Number of independent QTLs per chromosome found for BMI or BMI-related traits in table 5 of Rankinen 2005.
Figure S18. Correlation between association and linkage signals

Significant SNP effects for BMI and height were taken from the GIANT study\textsuperscript{50} and related to linkage signals from the 20,240 QISPs in this study (Figure 5). For each 1cM window (with 0.5cM overlaps) the sum of the LOD scores from the linkage analysis (y-axis) is plotted against the sum of the $-\log_{10} p$-values from the GIANT association (x-axis). The correlations for BMI and height are $r = -0.012$ and $r = 0.013$, respectively.
Table S1. Number of SNPs per cohort after pruning on LD and MAF

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Phenotype assessment method</th>
<th>Number of nuclear families</th>
<th>Number of unique individuals</th>
<th>Average number of SNPs per individual</th>
</tr>
</thead>
<tbody>
<tr>
<td>QIMR</td>
<td>Measured or self-reported</td>
<td>1935</td>
<td>6545</td>
<td>22655</td>
</tr>
<tr>
<td>Framingham Heart study</td>
<td>Measured</td>
<td>1605</td>
<td>4355</td>
<td>15747</td>
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<tr>
<td>TWINGENE</td>
<td>Self-reported</td>
<td>2722</td>
<td>5444</td>
<td>20193</td>
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<tr>
<td>Netherlands Twin Registry</td>
<td>Measured</td>
<td>1801</td>
<td>2556</td>
<td>20173</td>
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<tr>
<td>TwinsUK</td>
<td>Measured</td>
<td>1507</td>
<td>3014</td>
<td>20163</td>
</tr>
</tbody>
</table>

Table S2. Percentage variance explained by covariates on BMI and height

<table>
<thead>
<tr>
<th>Covariate</th>
<th>BMI</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>8.04</td>
<td>0.11</td>
</tr>
<tr>
<td>Sex</td>
<td>0.74</td>
<td>40.74</td>
</tr>
<tr>
<td>Year of birth</td>
<td>0.12</td>
<td>0.23</td>
</tr>
<tr>
<td>Cohort</td>
<td>3.67</td>
<td>5.86</td>
</tr>
<tr>
<td>Age squared</td>
<td>1.69</td>
<td>0.59</td>
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<tr>
<td>Age x sex</td>
<td>0.01</td>
<td>0.23</td>
</tr>
<tr>
<td>Age squared x sex</td>
<td>0.09</td>
<td>0.55</td>
</tr>
</tbody>
</table>

*aVariances were calculated from linear model analysis in which all covariates listed were fitted jointly.*