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Consort appendix DPP

Diabetes Prevention Program (DPP) Metabochip Genotyping Data Quality Control Methods

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Diabetes Prevention Program (DPP) Metabochip genotyping data was generated by the Genetic Analysis Platform (GAP) at the Broad Institute. Since some samples had DNA re-extracted to obtain enough amount for whole-genome amplification, two sets of genotyping experiments were performed. 2,976 samples in set1 and 388 samples in set2 passed, while 159 samples in set1 and 114 samples in set2 failed. Each set contained a PED and a MAP file. These files were loaded in PLINK software and two data sets were merged to create BED, FAM, and BIM files. All quality control tests were run in PLINK and R.

196,947 SNPs and 3,364 individuals were analyzed in total. Of 196,947 SNPs, 247 SNPs were unmapped, 1,475 SNPs had no genotype calls and 18,181 SNPs were monomorphic. All SNPs were reported on the positive strand of assembly B36.

14 samples were filtered for discrepant sex information by the sex check test. Individual and SNP (locus) call rate lists were generated. Missingness rates were higher than 80% for 24,487 SNPs, which were discarded. No individual was discarded for a high missingness rate.

A concordance test was run against data from a previously genotyped Illumina Oligonucleotide Pool Array (OPA) (sharing 108 SNPs and 3,271 individuals). The overall concordance rate was 98.80%. To check whether the Metabochip or the OPA genotyping run was the reason for instances of low concordance rate, previously genotyped Sequenom data was introduced to run the tests against the two Illumina arrays (Metabochip and OPA). The Metabochip and Sequenom shared 43 SNPs and 3,350 individuals: the concordance rate was 98.49%. The OPA and Sequenom shared 12 SNPs and 3,568 individuals: the concordance rate was 99.20%. Additional concordance tests were run on the individual level: there were 77 samples on Plate 11 in the Metabochip with <95% concordant rate. Further investigation revealed that this was due to a flipped plate. The concordance rate was recalculated after plate genotype re-flipping against a merged dataset of the OPA and Sequenom (containing 144 SNPs and 3,350 Individuals). It rose to 99.88%. There was only one individual with <95% concordance rate (78 discordant counts), which was excluded.

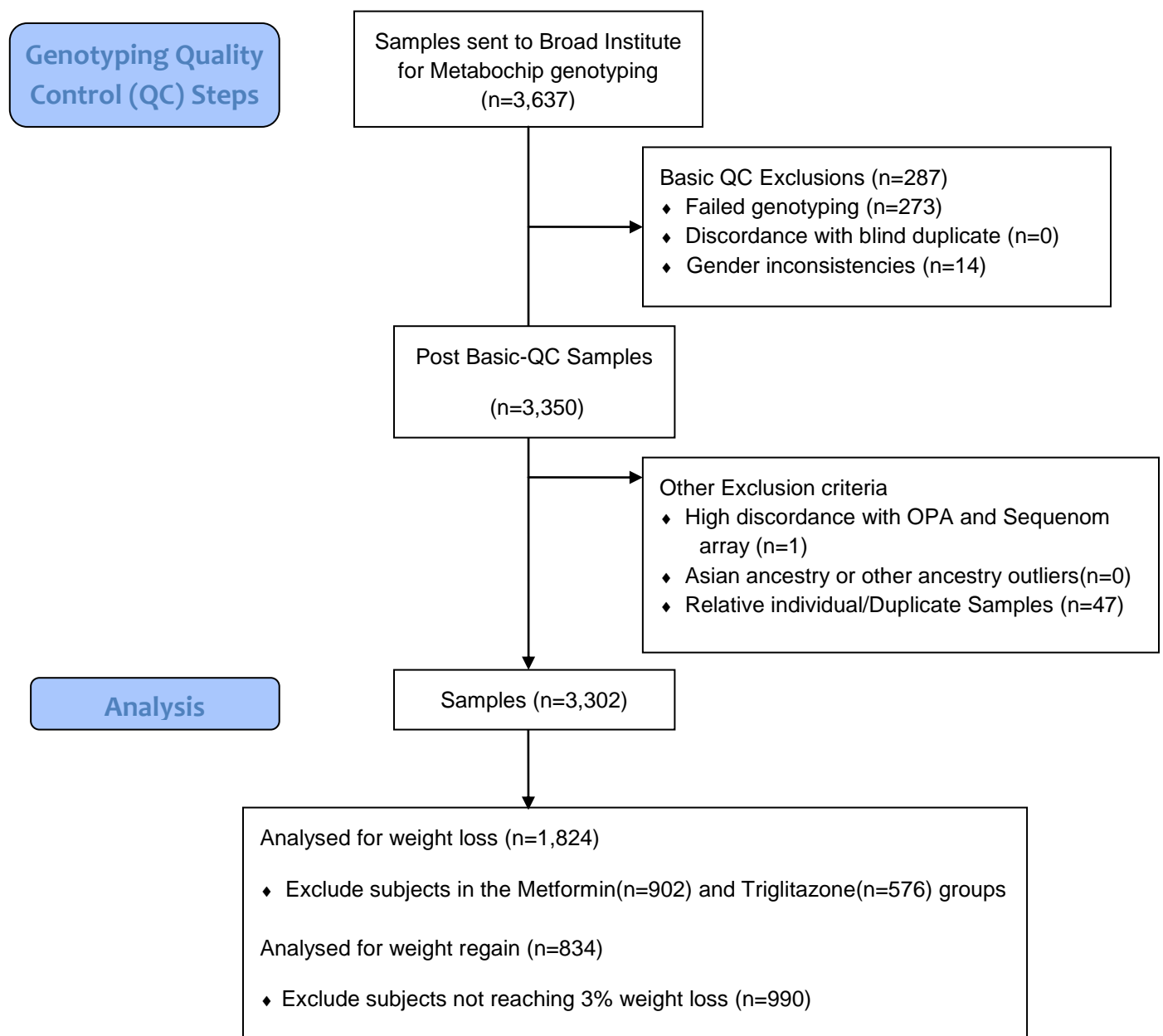
Ancestry/kinship information was unknown. Linkage disequilibrium (LD) test of SNPs on each chromosome (excluding X, Y, and mitochondrion) was run to prune out redundant SNPs in LD with prune-in SNPs. The inbreeding coefficient test was run on the pruned samples to check for sample contamination. This estimate argued against any contamination. To reduce computational size, total samples were split into 22 files, each file containing 150 samples. Identity by State (IBS) distance between individuals was computed by using pruned SNPs within each file. The results of each file were merged into one. The distribution of log₁₀ PI hat (which is an estimate that quantifies the genetic distance between two individuals) was continuous. Under the random mating population hypothesis for the whole study, PI hat of 58 pairs of relationships was higher than 0.5, which means the relationships were closer than parent-offspring and full-siblings. Further investigation revealed the presence of sample duplication for a subset of samples, due to a second shipment sent to the Broad Institute after an additional round of consents for genetic investigation: a total of 47 samples were filtered in this step.

SUPPLEMENTARY DATA

Hardy-Weinberg test was run and distribution plots for log₁₀ Hardy-Weinberg Equilibrium (HWE) *P* value were created for each ethnic group. After merging excluded SNP counts of all groups, a $P < 10^{-7}$ was picked as HWE *P*-value criterion for each ethnicity. 668 SNPs (0.39%) were on the excluding list.

Finally clean Metabochip data contained 171,792 SNPs and 3,302 individuals (1,090 males, 2,212 females). After filtering, IBS distance was re-computed. Principal Component Analysis (PCA) or Multidimensional Scaling (MDS) analysis was performed on the genome-wide IBS pairwise distances in conjunction with complete linkage clustering of individuals. ANOVA statistical analysis was performed to analyze from PC1 to PC10 on plating effects and the Wilcoxon rank sum test was performed on gender and set effects. No significant technical errors were found.

CONSORT Diagram for Diabetes Prevention Program Metabochip Experiment



Consort appendix LookAHEAD

Genotyping and Quality Control

A total of N=4,322 Look AHEAD experimental samples were sent to CIDR for genotyping. In addition to these samples, we also genotyped 35 unrelated HapMap samples (N=15 for CEU, N=4 for CHB, N=4 for JPT, N=3 for MEX, and N=9 for YRI) on the MetaboChip to serve as anchors in analyses of genetic ancestry. We removed samples that failed genotyping (>5% missing data), blind duplicate samples, samples displaying gender inconsistencies, and samples displaying a high degree of discordance with genotyping available from the IBC CVD array (11). This reduced the available sample size to 4,258.

To address issues related to population structure and latent familial clustering, we first created a subset of MetaboChip SNPs (177,142 out of 196,725) after removing non-autosomal SNPs, SNPs that could not be reliably mapped to the reference genome (see annotation information available at <http://www.sph.umich.edu/csg/kang/MetaboChip/>), SNPs in known regions of long-range linkage disequilibrium (LD) in European populations (12), SNPs with high discordance compared to genotyping available on the IBC CVD array, and SNPs with a high proportion of missing data (>5%). We then pruned this set of SNPs to create a final set of SNPs (67,592) in relative linkage equilibrium using the pair-wise pruning procedure (--indep-pairwise) implemented in *PLINK* (13), assuming a r^2 threshold of 0.2, a window size of 100 SNPs, and shifting the window 25 SNPs at a time.

Using the LD-pruned subset of SNPs, we then conducted a multi-dimensional scaling (MDS) analysis using *KING* (14). We removed samples of Asian ancestry (due to a small number of participants in the Look AHEAD trial) and outlying samples based on the MDS analysis, reducing the sample size to 4,121 (Supplementary Figure 1). We then estimated European, West African, and Native American admixture proportions (K=3) using *ADMIXTURE* (15), using these estimates within *REAP* (16) to estimate pair-wise kinship coefficients accounting for the multi-ethnic structure of the Look Ahead population (Supplementary Figure 2). Based on the kinship coefficient estimates from *REAP*, we then created an unrelated subset of participants using the Pedigree Reconstruction and Identification of a Maximum Unrelated Set (*PRIMUS*) software program (17), based on a kinship coefficient threshold of 0.025. The familial relationship pruning procedure reduced the available sample from 4,121 individuals to the final genetic sample size of 4,016 unrelated individuals.

When merging the genotypic and phenotypic data, we lost another 20 subjects due to missing outcome data, resulting in an analytic sample of N=3,906 individuals.

CONSORT Diagram for Look Ahead Metabochip Experiment

