Higher mitochondrial DNA copy number is associated with metformin-induced weight loss

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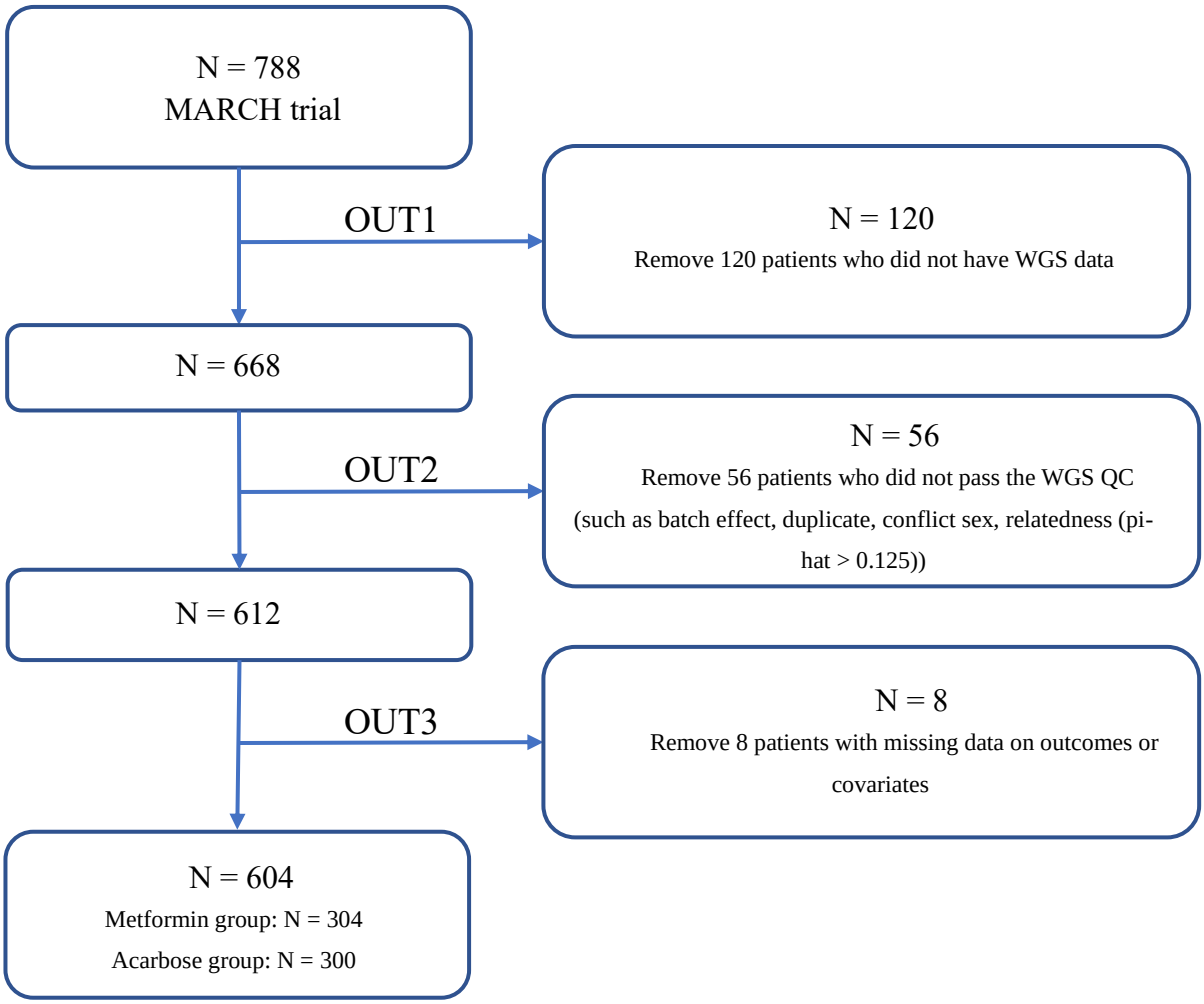
Supplementary Figures

Supplementary Figure 1. Flow chart of quality control of the study population



Supplementary Tables

Supplementary Table 1. The Genome characteristics of two drug treatment group

Baseline characteristics	Metformin treatment (N = 304)	Acarbose treatment (N = 300)	P
Average coverage of autosomal DNA	31.79 (5.79)	31.42 (3.45)	0.329
Average coverage of mtDNA	1785.64 (522.29)	1778.77 (539.29)	0.874
Raw estimated mtDNA-CN	112.69 (28.50)	113.35 (32.62)	0.791

Data are reported as mean (SD); P: the difference in baseline characteristics between metformin and acarbose treatment group.

Supplementary Table 2. Univariate linear regression between mtDNA-CN and gender, age, and blood cells

Baseline Characteristics	Beta (95% CI)	P value
Gender (Female)	0.09 (0.05 – 0.12)	<0.001
Age (years)	-0.82 (-1.54 – -0.1)	0.026
Platelets count (×109 cells/L)	10.9 (5.85 – 15.94)	<0.001
White blood cell count (×109 cells/L)	-0.14 (-0.27 – -0.01)	0.04
Red blood cell count (×109 cells/L)	-0.08 (-0.11 – -0.04)	<0.001

All models were adjusted for the batch effect. The effect sizes were expressed as beta (95% confidence interval).

Supplementary Table 3. Baseline characteristics of two drug treatment groups and the association between baseline characteristics and standardized mtDNA-CN

Baseline characteristics	Metformin treatment (N = 304)	Acarbose treatment (N = 300)	P*	Beta (95% CI) †	P †
Standardized mtDNA-CN	0.02 (0.96)	-0.03(1.04)	0.470	–	–
Demographic characteristics					
Male (%)	183 (60.20%)	185 (61.7%)	0.814	0 (-0.08–0.08)	0.923
Age (year)	50.46 (9.14)	50.33 (8.98)	0.860	0 (-0.08–0.08)	0.943
Weight (kg)	70.07 (10.69)	70.54 (10.45)	0.589	0.08 (0–0.16)	0.063
Height (cm)	165.56 (7.96)	165.65 (8.05)	0.889	0.05 (-0.03–0.13)	0.242
BMI (kg/m2)	25.63 (2.50)	25.78 (2.57)	0.470	0.07 (-0.01–0.15)	0.074
Clinical characteristics					
Systolic blood pressure (mm Hg)	123.04 (12.59)	122.93 (12.27)	0.909	0.02 (-0.06–0.1)	0.595
Diastolic blood pressure (mm Hg)	78.75 (7.93)	79.58 (8.45)	0.214	0.03 (-0.05–0.11)	0.464
LDL cholesterol (mmol/L)	3.00 (0.88)	3.08 (0.83)	0.238	0.02 (-0.06–0.1)	0.67
HDL cholesterol (mmol/L)	1.21 (0.27)	1.24 (0.28)	0.362	0 (-0.08–0.08)	0.941
Total cholesterol (mmol/L)	5.19 (1.01)	5.19 (0.98)	0.940	0.02 (-0.06–0.1)	0.624
Triglycerides (mmol/L)	1.92 (1.38–2.76)	1.71 (1.23–2.52)	0.055	0.05 (-0.03–0.13)	0.2
Glycemic characteristics					
HbA1c (%)	7.50 (1.13)	7.37 (1.08)	0.149	0.02 (-0.06–0.1)	0.573
Fasting blood glucose (mmol/L)	8.37 (1.40)	8.19 (1.38)	0.121	-0.02 (-0.1–0.06)	0.698

The data are reported as mean (SD), median (IQR) or n (%);

*: Baseline characteristics difference between metformin and acarbose arms;

†: The association between baseline characteristics and standardized mtDNA-CN in 604 participants. The effect sizes were expressed as Beta (95% confidence interval).

Supplementary methods

Whole genome sequencing and quality control

Genomic DNA was extracted from blood at baseline, and it was sequenced by Berry Genomics Co., Ltd. according to the standard protocols of NovaSeq 6000 platform. The sequencing reads were paired-end (2 × 150 nt) for each sample and the target depth is 30×. Sequencing reads quality was first checked with FastQC v0.11.3 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc). Adaptor sequences and low quality bases were removed with Trimmomatic v0.36 (1). The remaining reads were mapped to human reference genome assembly 38 (Cambridge Reference Sequence for mitochondrial genome) with BWA-MEM v0.7.15 (2). Picard was used to sort the BAM files and mark duplicates. Mapping quality was check by qualimap v2.1.2 (3). Indels were realigned and bases were recalibrated according to GATK v3.7 (4). Mosdepth v0.3.0 (5) was used to obtain the average coverages for autosomal DNA. In order to exclude the influence of nuclear integrations of mitochondrial sequences (NUMTs), reads aligning to the mitochondrial genome were extracted from each BAM file and analyzed using MToolBox v1.2.1 (6) to obtain the more accurate coverages of mitochondrial genome.

Measures of mtDNA Copy Number

The raw mtDNA-CN was calculated as the ratio of the average coverage of the mitochondrial genome and the autosomal genome. This approach has been used previously (7–10). In short, this approach assumes that genomic regions of equal ploidy

should be sequenced to comparable depth. Considering that the autosomal nuclear genome is at a fixed copy number in a normal human cell, the ratio of average coverage of the mitochondrial and nuclear genomes could be used as a rough proxy of mtDNA ploidy relative to a diploid standard (10).

$$\text{mtDNA copy number} = \frac{\text{mtDNA average coverage}}{\text{Autosomal DNA average coverage}} \times 2$$

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