Genetic Association Study of Common Mitochondrial Variants on Body Fat Mass

Tie-Lin Yang1, Yan Guo1*, Hui Shen2, Shu-Feng Lei2, Yong-Jun Liu2, Jian Li2, Yao-Zhong Liu2, Na Yu2, Jia Chen2, Ting Xu2, Yu Cheng2, Qing Tian2, Ping Yu2, Christopher J. Papasian2, Hong-Wen Deng2,3*

1 Key Laboratory of Biomedical Information Engineering of Ministry of Education, and Institute of Molecular Genetics, School of Life Science and Technology, Xi’an Jiaotong University, Xi’an, Shaanxi, People’s Republic of China, 2 School of Public Health and Tropical Medicine, Tulane University, New Orleans, Louisiana, United States of America, 3 Institute of Bioscience and Biotechnology, School of Science, Beijing Jiaotong University, Beijing, People’s Republic of China

Abstract

Mitochondria play a central role in ATP production and energy metabolism. Previous studies suggest that common variants in mtDNA are associated with several common complex diseases, including obesity. To test the hypothesis that common mtDNA variants influence obesity-related phenotypes, including BMI and body fat mass, we genotyped a total of 445 mtSNPs across the whole mitochondrial genome in a large sample of 2,286 unrelated Caucasian subjects. 72 of these 445 mtSNPs passed quality control criteria, and were used for subsequent analyses. We also classified all subjects into nine common European haplogroups. Association analyses were conducted for both BMI and body fat mass with single mtSNPs and mtDNA haplogroups. Two mtSNPs, mt4823 and mt8873, were detected to be significantly associated with body fat mass, with adjusted P-values of 4.94 x 10^-6 and 4.58 x 10^-6, respectively. The minor alleles mt4823 C and mt8873 A were associated with reduced fat mass values and the effect size (β) was estimated to be 3.52 and 3.18, respectively. These two mtSNPs also achieved nominally significant levels for association with BMI. For haplogroup analyses, we found that haplogroup X was strongly associated with both BMI (adjusted P = 8.31 x 10^-3) and body fat mass (adjusted P = 5.67 x 10^-3) Subjects classified as haplogroup X had lower BMI and fat mass values, with the β estimated to be 2.86 and 6.03, respectively. Our findings suggest that common variants in mitochondria might play a role in variations of body fat mass. Further molecular and functional studies will be needed to clarify the potential mechanism.

Introduction

All nucleated cells contain mitochondria, organelles whose most conspicuous roles are to produce ATP through respiration, and to regulate cellular and energy metabolism [1]. Interestingly, mitochondria are the only organelles of human cells, besides the nucleus, that contain their own DNA (mtDNA). Human mtDNA is a 16,569-bp, double-stranded circular molecule that contains 37 genes, and recent studies have demonstrated a relationship between mtDNA variants and several human complex diseases. Specifically, common variants in mtDNA have been shown to contribute to a variety of neurological and metabolic diseases [1], including diabetes [2,3], Alzheimer’s [4], and Parkinson’s [5] disease.

Obesity is a common and highly heritable disease in humans [6], whose prevalence has increased steadily in the US. If current trends continue, it is anticipated that over 50% of American adults will be obese by 2030, and that nearly 20% of total US health-care expenditures will be directed to obesity-associated diseases [7]. Obesity can be viewed as a disease of energy imbalance in which energy intake exceeds energy expenditure, resulting in excessive fat storage [8]. Given the essential function of mitochondria in energy metabolism, the potential contribution of mitochondrial dysfunction to cellular energy imbalances, metabolic disorders, and obesity, has been the subject of several recent studies [9,10]. For example, Pietiläinen et al. [9] found that mitochondrial energy metabolism pathways were down-regulated in obese twins in a study of 14 pairs of obesity discordant twins. Wurtman et al. [10] found a significant correlation between mitochondrial ATP production and age-related body mass index (BMI) by researching 172 children investigated for a suspected disorder of oxidative phosphorylation (OXPHOS). Moreover, recent population-based association studies investigating the relationship between common mtDNA variants and obesity found some nominally significant signals [11], suggesting that genetic variants in mtDNA might play an important role in susceptibility to obesity.

In this study, we performed genetic association tests to systematically examine the relationship between common mtDNA variants and obesity-related phenotypes, including body fat mass and BMI. We genotyped a total of 445 mitochondrial single...
nucleotide polymorphisms (mtSNPs) across the whole mitochondrial genome in a large sample of 2,286 unrelated Caucasian subjects. Of these 445 mtSNPs, 72 passed quality control, and these 72 mtSNPs were used to test for associations with BMI or body fat mass. Furthermore, since human mtDNA usually exists in haplogroup forms, we further classified subjects into nine common European haplogroups previously defined by Torroni et al. [12], based on specific mtSNPs throughout the mitochondrial genome. We then conducted association analyses between these haplogroups and BMI or body fat mass.

Results

The basic characteristics of the study sample set are summarized in Table 1. We first tested for associations between 72 mtSNPs and BMI or body fat mass. We summarized the major association results with BMI and body fat mass in Table 2. Data for 6 mtSNPs are presented, five of which showed nominally significant levels ($P<0.05$) of association with BMI (Table 2). However, none of those remained significant after adjustment for multiple testing. Five mtSNPs showed nominally significant levels ($P<0.05$) of association with body fat mass (Table 2). After adjustment for multiple testing, two mtSNPs, mt4823 and mt8873 were still significant, with adjusted $P$ values of $4.94 \times 10^{-3}$ and $4.58 \times 10^{-2}$, respectively. The minor allele C of mt4823 and allele A of mt8873 were both associated with reduced fat mass values with the effect size ($\beta$) estimated to be 3.52 and 3.18, respectively. These two mtSNPs also achieved nominally significant levels of association with BMI (Table 2). In order to validate our results, we randomly split the total sample in half, and re-performed association tests between mt4823 and mt8873 and body fat mass. These two mtSNPs remained consistently significant in both random samples (Sample 1: $P = 3.12 \times 10^{-3}$ for mt4823, $P = 1.41 \times 10^{-2}$ for mt8873; Sample 2, $P = 7.46 \times 10^{-3}$ for mt4823, $P = 4.05 \times 10^{-2}$ for mt8873).

For haplogroup analyses, all nine common European haplogroups were observed in our sample (Table 3). 95.7% of subjects belonged to these haplogroups, and the frequency of each haplogroup was consistent with the findings of previous studies of similar North American populations [5,12] (Table 4). After association tests, one haplogroup (X) showed a strong signal with BMI (adjusted $P = 8.31 \times 10^{-5}$) and body fat mass (adjusted $P = 5.67 \times 10^{-3}$). Subjects classified as haplogroup X had significantly lower BMI and fat mass values compared to subjects who did not belong to haplogroup X, and the $\beta$ was estimated to be 2.86 and 6.05, respectively. Considering the two significant SNPs we identified (mt4923 and mt7029), we further re-constructed haplogroup X including these two mtSNPs. The newly constructed haplogroup X captured by mt4923, mt8873, mt7029, mt10399 and mt14471, was in high linkage disequilibrium (LD) with the original haplogroup X ($r^2 = 0.87$). The newly constructed haplogroup X was also significantly associated with BMI and body fat mass, with adjusted $P$ values of $1.92 \times 10^{-5}$ and $8.51 \times 10^{-4}$, respectively. In order to validate our results we, again, randomly split the total sample in half, and re-performed association tests between haplotype X, body fat mass, and BMI. Results in the divided sample were still significant for body fat mass (Sample 1: $P = 2.56 \times 10^{-5}$, Sample 2: $P = 9.65 \times 10^{-6}$). For BMI, the $P$ value was significant in sample 1 ($P = 4.23 \times 10^{-5}$), and marginally significant in sample 2 ($P = 5.58 \times 10^{-2}$).

Discussion

In the current study, in which we examined associations between body fat mass or BMI with mtSNPs and mtDNA haplogroups, we identified two mtSNPs, mt4823 and mt8873, that were significantly associated with body fat mass after adjusting for multiple testing. These two mtSNPs also achieved nominally significant levels for association with BMI, but no significant association was detected between mtSNPs and BMI after adjusting for multiple testing. Our results also demonstrated that haplogroup X was significantly associated with both with BMI and body fat mass.

Our findings regarding BMI are largely consistent with the results of Saxena et al. [11] who detected nominal signals of association between BMI and specific mtSNPs in mitochondrial genes, including ND1, ND2, ND3 and ATPase6; after adjusting for multiple testing, these associations disappeared. Saxena et al. [11] also found a nominally significant association between haplogroup X and BMI (one-side $P$ value is 0.049). Thus, the potential link between haplogroup X and body fat mass is quite intriguing.

Although BMI is widely used as an index to identify overweight individuals and define obesity due to its ease of measurement, BMI cannot always distinguish obese people from large framed people. Sometimes, genetic association tests might be underpowered to detect obesity related genes using BMI independently. While using both BMI and body fat mass as response variable one may observe more interesting signals. This view was also supported by other studies. For example, the CTNMBL1 gene was recently implicated in the development of obesity in a genome-wide association study using both BMI (most significant SNP: $P = 2.69 \times 10^{-7}$) and body fat mass ($P = 4.99 \times 10^{-6}$) as obesity phenotypes [8,13]. However, if BMI was investigated independently, the signal didn’t achieve the genome-wide significance level (after multiple testing: $P = 0.06$). Therefore, BMI cannot be complemented by body fat mass, to comprehensively characterize obesity status.

The mt8873 A/G SNP is a nonsynonymous SNP, that causes an amino acid substitution of Phenylalanine (Phe) to leucine (Leu). The mt8873 SNP is located in the ATPase6 gene (encodes ATP synthase F0 subunit 6), which is essential for ATP synthase function. ATPase6 is a key component of the proton channel, and plays a direct role in the translocation of protons across the mitochondrial membrane. Mutations in ATPase6 have been implicated in several human diseases [14,15,16]. For example, two children found to carry an inherited mutation in the ATPase6 gene suffered from mild encephalopathy, and became obese following a period of normal postnatal growth [17]. Using Escherichia coli as a model, Ogilvie et al. [18] found that missense mutations in the ATPase6 gene can affect the ability of the enzyme to pump protons and synthesize ATP. Therefore, the mechanism by which mt8873 A/G affects obesity risk might be through alterations in the function of ATP synthase.

Table 1. Basic characteristics of the study subjects.

|                     | Total sample |
|---------------------|--------------|
| Number              | 2,286        |
| Gender (Males/Females) | 558/1,727   |
| Age (years)         | 51.37 (13.76) |
| Weight (kg)         | 75.27 (17.54) |
| Height (cm)         | 166.35 (8.47) |
| BMI (kg/m²)         | 27.14 (5.75) |
| Body fat mass (kg)  | 24.17 (10.63) |

Note: Data are shown as mean (standard deviation, SD.).

doi:10.1371/journal.pone.0021595.t001
The mt4823 SNP is located in the NADH dehydrogenase 2 gene (ND2), which is involved in the respiratory chain system and belongs to complex I of the mitochondrion. The mt4823 C/A is a synonymous change, which may act as a surrogate marker. The effect might be mediated through nearby SNPs in LD with mt4823. An additional explanation for the pathological mechanism of the mt4823 is that mt4823C and mt4823A may differentially affect OXPHOS. However, the potential mechanisms by which these SNPs impact obesity related phenotypes must be confirmed through further functional analyses.

A potential limitation of our study is that our findings are still unsubstantiated due to a lack of replication in other independent large samples, especially for haplogroup X that has a low frequency (0.01). However, we have cross-validated our results in sub-samples established by randomly dividing our sample in half. Additional support for our findings come from the study of Saxena et al. [11], suggesting that our findings are certainly worthy of follow-up. We intend to recruit additional subjects so that we can perform replication analyses in an independent sample. Moreover, we are hopeful that publication of our findings will facilitate replication analyses by other groups.

In conclusion, we identified two mtSNPs (mt4823 and mt8873) and mtDNA haplogroup X as markers that were significantly associated with reduced body fat mass. Our findings suggested that common variants in mitochondrial DNA play a role in variations of body fat mass. Further studies are warranted to explore the generality of our findings, and to determine the mechanisms underlying the risk of obesity.

### Materials and Methods

#### Study Subjects

The study was approved by the local institutional review boards or the office of research administration of all participating institutions. After signing an informed consent form, all subjects completed a structured questionnaire addressing anthropometric variables, lifestyles, and medical history. The sample contained 2286 unrelated adults, who were randomly identified from our established and expanding database currently containing more than 10,000 subjects. All of the chosen subjects were Caucasians of Northern European origin living in the Midwestern US. They were normal healthy subjects defined by a comprehensive suite of exclusion criteria [19]. Briefly, subjects with chronic diseases and conditions involving vital organs (heart, lung, liver, kidney and brain) and severe endocrine, metabolic or nutritional diseases that might affect fat metabolism were excluded from this study.

BMI was calculated as body weight (in kilograms) divided by the square of height (in meters). Weight was measured in light indoor clothing without shoes, using a calibrated balance beam scale, and height was measured using a calibrated stadiometer. Body fat mass was measured with Hologic 4500 DEXA machines. The short-term reproducibility (coefficient of variation) of BMI and fat mass measurements is on average 0.2 and 1.1%, respectively. Measurement of body fat mass by DEXA is considered to be highly accurate and gold standard. The correlation between BMI and fat mass was 0.85 ($P<0.01$) in this sample.

### Table 3. Classification of common European haplogroups.

| Haplogroup | C4217T | C7029T | A8252G | G10399A | C11720T | G12309A | A13709G | C14471T | G15219A | T16393C |
|------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| H          | ---    | C      | ---    | A      | ---    | ---    | ---    | ---    | ---    | ---    |
| I          | ---    | T      | A      | G      | ---    | ---    | G      | ---    | ---    | ---    |
| J          | ---    | T      | ---    | G      | ---    | ---    | A      | ---    | ---    | ---    |
| K          | ---    | T      | ---    | G      | ---    | ---    | G      | G      | ---    | ---    |
| T          | C      | T      | G      | A      | ---    | ---    | G      | ---    | ---    | ---    |
| U          | ---    | T      | ---    | A      | ---    | G      | ---    | ---    | ---    | ---    |
| V          | T      | T      | G      | A      | C      | ---    | ---    | ---    | A      | C      |
| W          | ---    | T      | A      | A      | ---    | A      | G      | ---    | ---    | ---    |
| X          | ---    | T      | ---    | A      | ---    | ---    | C      | ---    | ---    | ---    |

doi:10.1371/journal.pone.0021595.t003

---

### Table 2. Major association results of mtDNA variants with BMI and body fat mass ($P<0.05$).

| mtSNP  | Gene   | Alleles | Amino change | MAF          | $\beta_{BMI}$ | $\rho_{adjusted}$ | $\rho_{BMI}$ | $\rho_{body fat mass}$ | $\rho_{adjusted}$ | $\rho_{body fat mass}$ |
|--------|--------|---------|--------------|--------------|--------------|-------------------|--------------|------------------------|-------------------|------------------------|
| mt4823 | ND2    | C/A     | Val -> Val   | 0.042        | 5.58 x 10^{-3}| 4.01 x 10^{-1} | -1.36        | 6.86 x 10^{-5} | 4.94 x 10^{-3} | -3.52               |
| mt8873 | ATPase6| A/G     | Phe -> Leu   | 0.029        | 6.32 x 10^{-3}| 1.51 x 10^{-1} | -1.48        | 1.27 x 10^{-3} | 4.58 x 10^{-2} | -3.18               |
| mt16321| D-loop | A/G     | ---          | 0.014        | 4.03 x 10^{-3}| 1.45 x 10^{-1} | -2.90        | 4.13 x 10^{-3} | 9.91 x 10^{-2} | -4.81               |
| mt16141| D-loop | C/T     | ---          | 0.027        | 1.39 x 10^{-2}| 2.51 x 10^{-1} | -1.72        | 5.25 x 10^{-3} | 9.45 x 10^{-2} | -3.29               |
| mt12247| tRNA Ser| C/G     | ---          | 0.035        | 2.46 x 10^{-3}| 1.00           | -0.75        | 3.82 x 10^{-2} | 5.50 x 10^{-1} | -2.10               |
| mt11300| ND4    | C/T     | Thr -> Thr   | 0.077        | 2.91 x 10^{-2}| 4.19 x 10^{-1} | -0.92        | 1.34 x 10^{-3} | 7.45 x 10^{-1} | -1.21               |

doi:10.1371/journal.pone.0021595.t002
Statistical analyses

Table 4. Association results of common European haplogroups with BMI and body fat mass.

| Haplogroup | $P_{BMI}$ | $P_{adjusted}$ | $P_{BMI}$ | $P_{body fat mass}$ | $P_{adjusted}$ | $P_{body fat mass}$ | frequency |
|------------|-----------|----------------|-----------|---------------------|----------------|---------------------|-----------|
| H          | 0.440     | NS             | -0.203    | 0.533               | NS             | -0.271              | 0.437     |
| J          | 0.975     | NS             | -0.093    | 0.876               | NS             | 0.120               | 0.086     |
| U          | 0.434     | NS             | -0.307    | 0.608               | NS             | -0.279              | 0.164     |
| K          | 0.412     | NS             | -0.484    | 0.984               | NS             | -0.017              | 0.059     |
| I          | 0.803     | NS             | 0.086     | 0.572               | NS             | 0.806               | 0.027     |
| W          | 0.279     | NS             | 0.904     | 0.222               | NS             | 2.140               | 0.023     |
| T          | 0.391     | NS             | 0.206     | 0.625               | NS             | 0.353               | 0.099     |
| X          | $8.31 \times 10^{-4}$ | $8.31 \times 10^{-3}$ | -2.856     | $5.67 \times 10^{-2}$ | $5.67 \times 10^{-4}$ | -0.031       | 0.010     |
| V          | 0.682     | NS             | -0.289    | 0.625               | NS             | -0.446              | 0.052     |

Genotyping and quality control

Genomic DNA was extracted from whole human blood using a commercial isolation kit (Genuta systems, Minneapolis, MN, USA) following the standard protocol. Genotyping was carried out using the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix, Santa Clara, CA, USA), which includes 445 mtSNPs throughout the mitochondrial genome, according to the Affymetrix protocol. Briefly, approximately 250 ng of genomic DNA was digested with restriction enzyme NspI and StyI. Digested DNA was adaptor-ligated and PCR-amplified for each sample. Fragment PCR products were then labeled with biotin, denatured, and hybridized to the arrays. Arrays were then washed and stained using Phycoerythrin on Affymetrix Fluidics Station, and scanned using the GeneChip Scanner 3000 7G estimated by linear regression.

Supporting Information

Table S1 Properties of SNPs in mtDNA tested in this study.

Author Contributions

Conceived and designed the experiments: H-WD T-LY. Performed the experiments: HS S-FL NY JC TX YC PY. Analyzed the data: T-LY YG JL. Contributed reagents/materials/analysis tools: HS Y-JL Y-ZL QT. Wrote the paper: T-LY CJP.
References

1. DiMauro S, Schon EA (2003) Mitochondrial respiratory-chain diseases. N Engl J Med 348: 2666–2668.
2. Chinnery PF, Elliott HR, Patel S, Lambert C, Keers SM, et al. (2005) Role of the mitochondrial DNA 16184-16193 poly-C tract in type 2 diabetes. Lancet 366: 1650–1651.
3. Pouhout J, Luan J, Macaulay V, Herrnings S, Mitchell J, et al. (2002) Type 2 diabetes is associated with a common mitochondrial variant: evidence from a population-based case-control study. Hum Mol Genet 11: 1581–1583.
4. Chagnon P, Gee M, Filion M, Robitaille Y, Belouchi M, et al. (1999) Phylogenetic analysis of the mitochondrial genome indicates significant differences between patients with Alzheimer disease and controls in a French-Canadian founder population. Am J Med Genet 85: 20–30.
5. van der Walt JM, Nicodemus KK, Martin ER, Scott WK, Nance MA, et al. (2003) Mitochondrial polymorphisms significantly reduce the risk of Parkinson disease. Am J Hum Genet 72: 804–811.
6. Bell CG, Walley AJ, Froguel P (2005) The genetics of human obesity. Nat Rev Genet 6: 221–234.
7. Wang Y, Beydoun MA, Liang L, Caballero B, Kumanyika SK (2008) Will all Americans become overweight or obese? estimating the progression and cost of the US obesity epidemic. Obesity (Silver Spring) 16: 2323–2330.
8. Walley AJ, Asher JE, Froguel P (2009) The genetic contribution to non-syndromic human obesity. Nat Rev Genet 10: 431–442.
9. Pietilainen KH, Naukkarinen J, Rissanen A, Saharinen J, Ellonen P, et al. (2008) Global transcript profiles of fat in monozygotic twins discordant for BMI: pathways behind acquired obesity. PLoS Med 5: e51.
10. Wortmann SB, Zweers-van Essen H, Rodenburg RJ, van den Heuvel LP, de Vries MC, et al. (2009) Mitochondrial energy production correlates with the age-related BMI. Pediatr Res 65: 103–108.
11. Saxena R, de Bakker PI, Singer K, Mootha V, Burtt N, et al. (2006) Comprehensive association testing of common mitochondrial DNA variation in metabolic disease. Am J Hum Genet 79: 54–61.
12. Torroni A, Huoponen K, Francalacci P, Petruzi M, Morelli L, et al. (1996) Classification of European mtDNAs from an analysis of three European populations. Genetics 144: 1835–1850.