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Single nucleotide polymorphisms linked to mitochondrial uncoupling protein genes *UCP2* and *UCP3* affect mitochondrial metabolism and healthy aging in female nonagenarians

Sangkyu Kim¹, Leann Myers², Eric Ravussin³, Katie E. Cherry⁴, and S. Michal Jazwinski¹

¹Tulane Center for Aging and Department of Medicine, Tulane University Health Sciences Center, New Orleans, LA

²Department of Biostatistics and Bioinformatics, School of Public Health and Tropical Medicine, Tulane University Health Sciences Center, New Orleans, LA

³Pennington Biomedical Research Center, Baton Rouge, LA

⁴Department of Psychology, Louisiana State University, Baton Rouge, LA

Abstract

Energy expenditure decreases with age, but in the oldest-old, energy demand for maintenance of body functions increases with declining health. Uncoupling proteins have profound impact on mitochondrial metabolic processes; therefore, we focused attention on mitochondrial uncoupling protein genes. Alongside resting metabolic rate (RMR), two SNPs in the promoter region of *UCP2* were associated with healthy aging. These SNPs mark potential binding sites for several transcription factors; thus, they may affect expression of the gene. A third SNP in the 3'-UTR of *UCP3* interacted with RMR. This *UCP3* SNP is known to impact *UCP3* expression in tissue culture cells, and it has been associated with body weight and mitochondrial energy metabolism. The significant main effects of the *UCP2* SNPs and the interaction effect of the *UCP3* SNP were also observed after controlling for fat-free mass (FFM) and physical-activity related energy consumption. The association of *UCP2/3* with healthy aging was not found in males. Thus, our study provides evidence that the genetic risk factors for healthy aging differ in males and females, as expected from the differences in the phenotypes associated with healthy aging between the two sexes. It also has implications for how mitochondrial function changes during aging.

Keywords

healthy aging; frailty; energy metabolism; mitochondrial uncoupling; single nucleotide polymorphism

Corresponding author: Sangkyu Kim, PhD, Tulane Center for Aging and Department of Medicine, Tulane University Health Sciences Center, 1430 Tulane Ave., SL-12, New Orleans, LA 70112, Tel.: 504-988-3091; fax: 504-988-8835; skim5@tulane.edu.

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Introduction

RMR measures the energy demand for body functioning at rest. Normally, the amount of energy consumed for resting metabolism exceeds that for physical activity several-fold (Ruggiero and Ferrucci 2006). Both of these major forms of energy expenditure decline with increasing age (Kim and Jazwinski 2015). In nonagenarians, elevated RMR is associated with declining health, which is represented by increased FI₃₄, a frailty index based on 34 health variables (Kim et al. 2013). FI₃₄ is a reliable indicator of biological age, with a heritability estimate of 0.39 (Kim et al. 2013). Some factors show gender-specific associations with FI₃₄. In male nonagenarians, circulating creatine kinase (CK) is positively associated with FI₃₄; in female nonagenarians, fat mass (FM) and FFM are important contributors to healthy aging (Kim et al. 2014).

UCP2 and UCP3 (UCP2/3) are two of the five homologs of UCP1, the first UCP protein demonstrated to have a mitochondrial uncoupling function (Gimeno et al. 1997; Matthias et al. 2000). UCP2/3 share 57-59% amino-acid sequence identity with UCP1 (Krauss et al. 2005). UCP4 and UCP5 have much lower sequence identity with UCP1, and relatively less is known about the functions of these homologs (Ho et al. 2012; Kwok et al. 2010).

The original uncoupling function assigned to UCP1 involves dissipation of the proton motive force to thermogenesis (Rousset et al. 2004). Some protons in the mitochondrial intermembrane space are transferred to the matrix by proton carriers without participating in oxidative phosphorylation. The best known example of this proton 'leak' is dissipation of the proton motive force as heat in brown adipose tissue (BAT), where ATP synthase expression is relatively low but UCP1 expression is high (Klaus et al. 1991; Thomas and Palmiter 1997). Unlike UCP1 expression, UCP2/3 expression is not limited to BAT. *UCP3* mRNA and protein are abundant in skeletal muscle and heart tissues (Boss et al. 1997; Rousset et al. 2004). Because of translational control, *UCP2* protein is hardly detected in these tissues even though its mRNA is abundant (Velloso et al. 2009). Production of UCP2 protein can increase more than 10-fold under certain stress conditions, like fasting (Pecqueur et al. 2001). Thus, abundance of *UCP2/3* mRNA or protein in tissues other than fat suggests that thermogenesis may not be the function of these proteins.

Indeed, UCP2/3 have multiple roles. They affect mitochondrial energy generation, key metabolite use, insulin secretion, and generation of reactive oxygen species (ROS). Thymocytes isolated from *UCP2*-knockout mice show lower proton leak, higher mitochondrial membrane potential, and higher cellular ATP concentrations, compared with those from wild-type mice (Krauss et al. 2002). These uncoupling proteins are carriers of such important metabolites as fatty acids and mitochondrial four-carbon metabolites (Jaburek et al. 1999; Vozza et al. 2014). UCP2 negatively regulates insulin secretion from pancreatic β cells by decreasing the ATP/ADP ratio (Zhang et al. 2001). *UCP3* protein levels are lower in type 2 diabetic patients (Schrauwen et al. 2001). Besides their effects on metabolic processes, UCP2/3 can lower oxidative stress by reducing ROS generation from incomplete reduction of oxygen, and oxidative stress can be an important regulator of cell survival and aging (Mookerjee et al. 2010). Importantly, polymorphisms in *UCP2/3* are linked to RMR in humans (Bouchard et al. 1997).

The functions of *UCP2/3* in metabolism, bioenergetics, and ROS generation point to these genes as potential genetic risk factors for healthy aging. In this report, we examined association of the *UCP2/3* genes with healthy aging by evaluating the relationship between single-nucleotide polymorphisms (SNP) in *UCP2/3* and FI_{34} after adjustment for relevant covariates.

Materials and methods

Participants

Study participants were 67 Caucasian nonagenarians from the Louisiana Healthy Aging Study (LHAS) (Jazwinski et al. 2010; Kim et al. 2014), 37 females aged from 90 to 98 and 30 males aged from 90 to 97. Ages were based on demographic questionnaires and documentary evidence. All participants provided informed consent according to protocols approved by the respective Institutional Review Boards.

Genotyping and SNPs in *UCP2* and *UCP3*

The Illumina GoldenGate assay was used for SNP genotyping (Jazwinski et al. 2010). SNPs were selected according to the Illumina Assay Design Tool, and quality control measures were applied using Illumina GenomeStudio. For SNP and sample clustering, the 10th percentile GenCall score was set at 0.4. Samples with call rates below 90% were excluded. *UCP2/3* are located in tandem on chromosome 11, and the three SNPs analyzed in this study are polymorphic and in Hardy-Weinberg equilibrium (Table 1). To examine the additive genetic effect of each SNP in the multiple linear regression analysis, its genotype was numerically recoded according to the copy number of the minor allele (0, 1, 2).

Data management and analysis

Ethnic origin of each participant was inferred genetically using Alu genotypes, and Caucasians were included in data analysis to avoid population confounding (Jazwinski et al. 2010). The summary statistics of the study variables for the 67 subjects are shown in Table 1. Details of data collection and calculation and characterization of FI_{34} were described elsewhere (Kim et al. 2013). FI_{34} is normally distributed among the nonagenarians. Measurements of body and metabolic parameters used in this study were described previously (Frisard et al. 2007). FFM is the total body weight multiplied by the proportion of non-fat mass, measured using dual-emission X-ray absorptiometry (DXA). RMR was measured by indirect calorimetry during rest in the fasting state. The energy expenditure summary index (EESI) is one of the three indices of the Yale Physical Activity Survey (Dipietro et al. 1993). It is calculated by multiplying the time for an activity by an intensity code and summing the product over all the activities for a week. All the statistical analyses were performed using R (R_Core_Team 2014).

SNP annotation

Linkage disequilibrium (LD) blocks for *UCP2/3* were examined using Haploview with the standard color scheme using D'/LOD values (Barrett 2009). Images summarizing genomic features around *UCP2/3* were obtained using the UCSC Genome Browser (Rosenbloom et

al. 2015) and RegulomeDB (Boyle et al. 2012), based on the Human Feb. 2009 (GRCh37/hg19) Assembly.

Results

Characteristics of the nonagenarian sample

Male and female nonagenarians in our study sample differed in FI_{34} , RMR, and FFM (Table 2). The mean FI_{34} of the females was significantly higher than that of the males ($p = 0.0022$), indicating that females were overall less healthy than males of the same age group. On the other hand, mean RMR and FFM values were much higher in males ($p < 0.001$). EESI means didn't differ, probably because EESI sums up mostly light or moderate physical activities in both genders.

Although RMR goes down with age overall (Kim and Jazwinski 2015), it goes up with FI_{34} among nonagenarians, which was observed after adjustment for FFM, FM, and age (Kim et al. 2014). This suggests increasing resting energy consumption with deteriorating health. Although this association of RMR with FI_{34} is common to both sexes, we found male-specific elevation of circulating CK levels and female-specific decline in FFM (Kim et al. 2014).

SNPs rs591758 and rs675547 are significant predictors of FI_{34}

The lower FFM in unhealthy female nonagenarians coupled to higher RMR is surprising. It suggests that features of energy metabolism, especially in muscle, may change in female nonagenarians as their health declines. To gain an understanding of these changes, we searched for genetic risk factors associated with these changes. Mitochondrial uncoupling proteins affect muscle energy metabolism in multiple ways. Therefore, we examined association of *UCP2/3* SNPs with FI_{34} with adjustment for RMR. SNP rs591758 is predicted to affect binding of transcription factors (Fig. 1). It is a variant in risk haplotypes associated with type 2 diabetes in Caucasian women (Hsu et al. 2008). SNP rs675547 is also predicted to affect transcription factor binding sites (Fig. 2). Both rs591758 and rs675547 are in an LD block (Block 1) covering *UCP2* (Fig. 3). SNP rs1626521, located in the 3' untranslated region of *UCP3* (Fig. 4), is associated with body weight and mitochondrial energy metabolism and affects expression of a reporter gene in an allele-specific manner (Acosta et al. 2015). Therefore, we chose these three SNPs (Table 1) to test for association with FI_{34} .

The *UCP2* SNP rs591758 was significantly associated with FI_{34} without affecting the significance and direction of association of RMR (Table 3). The additive genetic effect of the minor allele of rs591758 was positively associated with FI_{34} , indicating that an increase in the copy number of the minor allele is associated with unhealthy aging. Similar results were obtained with rs675547. These associations were observed only in the female group. On the other hand, no association of the *UCP3* SNP rs1626521 with FI_{34} was seen; however, its inclusion in the model eliminated the significant association of the covariate RMR (Table 3). These results suggest that the *UCP2* variants have an effect on healthy aging independent of RMR, while the effect of the *UCP3* variant is closely related to it.

A significant interaction between rs1626521 and RMR

To evaluate our conclusions regarding the effects of the *UCP2/3* variants, we examined interactions of the SNPs with RMR (Table 4). There was no significant interaction of the two *UCP2* SNPs with RMR found. For the *UCP3* SNP, however, a significant interaction with RMR was detected ($p = 0.018$; Table 4). This indicates that the association of RMR with FI_{34} is inseparable from *UCP3* genotypes (Fig. 5).

Association of the *UCP2/3* SNPs in multiple covariate models

The models involving RMR alone did not adjust for other factors that may be related to the association of RMR with FI_{34} and thus affect the relationship between FI_{34} and the SNPs. FFM and EESI are two such likely factors (FFM is the body mass remaining after subtraction of FFM, which is obtained by multiplying total body weight by percent non-fat body mass determined by DEXA. Thus, they are complementary measures.) *UCP2/3* are expressed predominantly in tissues other than fat, especially in skeletal muscle, and FFM is associated with FI_{34} in the females, as stated earlier. Physical activity energy expenditure is related to skeletal muscle mass and function, and our studies have shown that it is related to FI_{34} (Kim and Jazwinski 2015). Therefore, we included FFM and EESI in multiple regression analyses (Table 5). In the models containing each of the individual *UCP2* SNPs, the association of the SNP and RMR with FI_{34} remained, while FFM and EESI showed the expected, significant association with FI_{34} . In the model with the *UCP3* SNP, the same relationships were apparent, except that no association of the SNP with FI_{34} was found, as before. Thus, both FFM and EESI were inversely associated with FI_{34} , indicating that muscle mass and physical activity are associated with healthy aging and that *UCP2* contributes as well.

We next examined the interactions between RMR and the *UCP2/3* variants in the presence of FFM and EESI, to determine whether they were affected by muscle mass and physical activity energy expenditure. The interaction between RMR and the *UCP3* SNP remained significant, while no interaction was detected for the *UCP2* SNPs as before (Table 6). Therefore, the models that take into account FFM and EESI confirm the association of *UCP2/3* SNPs with FI_{34} seen when only RMR is included.

Discussion

We examined three *UCP2/3* SNPs that are predicted or known to be functional for their association with FI_{34} . SNPs rs591758 and rs675547, which are linked to *UCP2*, were significantly associated with FI_{34} , whereas the *UCP3* SNP rs162651 was not (Tables 3 and 5). The *UCP2* SNPs showed the same allele specificity. An increase in the copy number of the minor allele was associated with an increase in FI_{34} of about 0.03 to 0.04. Based on the exponential accumulation of health deficits ($FI_{34} = 0.03 \cdot e^{(0.02 \cdot \text{age})}$) (Kim et al. 2013), an increase in age from 90 to 91 is expected to increase FI_{34} on average by 0.0037 in the population studied. Thus, the additive genetic effect of the *UCP2* SNPs is substantially greater than the annual chronological effect at age 90. However, this must be placed in context. The activity of *UCP2* occurs over a lifetime and not only for one year at advanced ages. In contrast to the *UCP2* SNPs, the *UCP3* SNP moderated the association of RMR with FI_{34} (Tables 4 and 6, Fig. 5), without an effect on its own. Thus, this differentiates the *UCP3*

SNP from the *UCP2* SNPs, which likely reflects functional differences of the gene products. All the associations were specific only to female nonagenarians.

Functions of the SNPs and healthy aging

UCP2 variants have been associated with various traits related to body mass and composition. *UCP2* negatively regulates insulin secretion by lowering the ATP/ADP ratio, implying a role in obesity and type 2 diabetes (Krauss et al. 2005; Rousset et al. 2004). A recent study highlights *UCP2* as a transporter of important metabolites of the Krebs cycle rather than as an uncoupling protein associated with thermogenesis (Voza et al. 2014). This study shows that *UCP2* negatively controls oxidation of acetyl-CoA by transporting four-carbon intermediates, such as oxaloacetate, out of mitochondria. This export of metabolic intermediates, assisted by proton gradients, lowers the redox potential, ATP/ADP ratio, and possibly ROS generation (Voza et al. 2014). Besides limiting glucose oxidation, *UCP2* can promote mitochondrial processing of other energy sources such as fatty acids and glutamine. In fact, *UCP2* can enhance fatty acid oxidation (Pecqueur et al. 2008) and possibly glutaminolysis, because its translation from mRNA is activated by glutamine (Hurtaud et al. 2007). Furthermore, overexpressed in many actively proliferating tumor cells, *UCP2* can promote energy production from aerobic breakdown of glucose to lactate (Ayyasamy et al. 2011), a phenomenon termed the Warburg effect (Warburg 1956). Thus, increased *UCP2* expression can alter cellular profiles of energy metabolism under certain physiological conditions.

Included in the same LD block, rs591758 and rs675547 are predicted to affect binding of transcription factors such as MyoD and the CCCTC-binding factor CTCF, respectively. Along with Myf6, MyoD belongs to a group of myogenic regulatory transcription factors (Anand et al. 1994; Cao et al. 2010). Indeed, MyoD can activate expression of *UCP2/3* in several cell lines, including differentiating myoblasts, with the expression level depending on the availability of additional cofactors (Kim et al. 2007). CTCF is a highly conserved, zinc-finger transcription factor with various effects on gene expression (Filippova et al. 1996). CTCF enhances the myogenic activity of MyoD (Delgado-Olguin et al. 2011), and these two proteins interact physically through chromatin looping (Battistelli et al. 2014). In this study, we found that the additive genetic effects of the minor alleles of these *UCP2* SNPs were positively associated with FI₃₄. The minor allele (G) of rs591758 corresponds to the last sequence position of the MyoD-binding motif (CANNTG; Fig. 3c). Therefore, assuming an active role of this MyoD motif in *UCP2* expression, the minor allele would promote binding of MyoD, activating *UCP2* expression. We speculate that increased expression of *UCP2* in the oldest-old could be a sign of altered cellular energy metabolism associated with unhealthy aging, similar to the Warburg effect in cancer cells. In other words, energy metabolism profiles may vary depending on the genotypes of the *UCP2* SNPs. A better understanding of the relationship between *UCP2* expression and healthy aging awaits experimental data on the functions of the SNPs and further characterization of the uncoupling protein.

The *UCP3* SNP rs1626521 is functional and associated with body weight and mitochondrial energy metabolism in skeletal muscle (Acosta et al. 2015). Other SNPs in *UCP3* are

associated with childhood obesity and hand-grip strength (Crocco et al. 2011; Dato et al. 2012; Musa et al. 2012). The LD block 2 in which rs1626521 belongs contains seven miRNA target sites as well as a missense variant rs8179179 (Fig. 4), increasing the likelihood of the functionality of the SNP. Expression of a reporter gene in the presence of the minor allele is significantly higher than in the presence of the major allele (Acosta et al. 2015). The interaction of the SNP with RMR indicates that the positive association of RMR with FI₃₄ depends on the minor allele of the SNP (Fig. 5). Specifically, an increase in RMR among the frail nonagenarian females accompanies an increase in UCP3 expression. It is unclear how exactly UCP3 expression relates to RMR in frail female nonagenarians. We speculate, however, that increased expression of UCP3 in the oldest-old with deteriorating health leads directly to elevated RMR without altering the essential characteristics of energy metabolism. Other studies have shown that an allele of a SNP (rs1800849) in the promoter region that drives higher UCP3 expression (Schrauwen et al. 1999) is associated with higher hand-grip strength (Crocco et al. 2011). However, this association was not significant in the nonagenarian sample. Furthermore, the female nonagenarians in this study displayed lower hand-grip strength. Thus, we suggest that increased UCP3 expression and associated increase in RMR compensates for the decline in health and related decline in muscle mass in female nonagenarians.

Oxidative damage

Another possibility is that the association of *UCP2/3* with FI₃₄ could be through their effects on mitochondrial ROS generation. The uncoupling proteins can protect cells from oxidative damage by reducing ROS production (Brand et al. 2004; Krauss et al. 2005). Superoxide even enhances proton conductance of skeletal muscle mitochondria by activating *UCP2* and *UCP3* (Echtay et al. 2002). This indicates operation of a coordinated feedback mechanism to regulate the mitochondrial proton leak and membrane potential. Mitochondrial membrane potential and ROS generation are highly correlated, and ROS generation is sensitive to changes in membrane potential; therefore, mild uncoupling through increased expression of *UCP2/3* can be an efficient way of controlling mitochondrial oxidative damage (Echtay 2007).

UCP genes are associated with human longevity (Rose et al. 2011). The importance of oxidative damage as a determinant of longevity is found in rodent studies. For example, longevity in rats is correlated with ROS production (Mookerjee et al. 2010). Mice lacking *UCP2* have shorter lifespans compared with the wild type, and mice lacking the superoxide dismutase 2 gene tend to live longer as the copy number of *UCP2* increases (Andrews and Horvath 2009). However, other studies show no effect of *sod2* on murine life span (Gallagher et al. 2000; Zhang et al. 2009). According to our current study, the minor alleles of the *UCP2/3* SNPs examined were correlated with increased gene expression, which is evident from the gene reporter assay with the *UCP3* SNP (Acosta et al. 2015). Because increased expression of *UCP2/3* corresponds to increased uncoupling, and increased uncoupling is expected to reduce ROS production, the minor alleles should be associated with healthier aging. On the contrary, we found the opposite: all three minor alleles of the *UCP2/3* SNPs were positively associated with FI₃₄. Therefore, we think that reduction in ROS generation, if any, through increased uncoupling may have a minor effect on healthy

aging. It is possible that the effect of the *UCP2/3* gene variants on longevity comes from combinations of several functions of these genes rather than the sole effect on oxidative stress (Rose et al. 2011).

Gender specificity

The association of *UCP2/3* with FI_{34} was specific to the female nonagenarians. The population study of rs1626521 by Acosta et al. (2015) involved both males and females, but mostly in their 30s. In our study, the largest gender difference was in FFM. The mean FFM of the male nonagenarians was about 40% higher than that of the female nonagenarians ($p < 0.0001$, Table 2). Because *UCP2/3* expression is most abundant in FFM such as heart and skeletal muscle, one would expect greater associations of these uncoupling genes with the healthy aging of males. Yet, we didn't find such associations in males. However, we noticed that RMR per unit FFM is significantly higher in the female nonagenarians than in the males (27.5 vs 24.6, $p < 0.0001$). Therefore, any change in FFM would have a greater effect on energy metabolism in the females than in the males. Thus, the genetic effect of *UCP2/3* on energy metabolism would be greater in the females.

A caveat to our conclusions is the sample size in this analysis. Although it begins with 67 nonagenarians, it then focuses on an examination of the females in this group who show a gender-specific association of FFM with healthy aging, by investigation of the impact of *UCP2/3* on this association. Despite the relatively small sample, post-hoc power analysis showed that the $1-\beta$ values of all the models described here exceed 0.8 at $n = 37$ and $\alpha = 0.05$.

In sum, we investigated the effect of *UCP2/3* on healthy aging in nonagenarians. We used two predicted functional SNPs in *UCP2* and an experimentally-validated functional SNP in *UCP3*. For each of the *UCP2* SNPs, the minor allele copy number was positively associated with an increase in FI_{34} . Unlike the *UCP2* SNPs, the *UCP3* SNP moderated the relationship between RMR and FI_{34} . The net effect of the *UCP3* SNP was the same as that of the *UCP2* SNPs: the minor allele was positively associated with FI_{34} . All these associations were specific to female nonagenarians. Our results provide evidence for the function of *UCP2/3* in healthy aging in nonagenarian females.

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b

Method	Location	Bound Protein	? Cell Type
ChIP-seq	chr11:73697912..73698196	FOXA1	HepG2
ChIP-seq	chr11:73697949..73698229	FOXA1	HepG2
ChIP-seq	chr11:73697949..73698239	FOXA2	HepG2

c

Figure 1a

Method	Location	Motif	? Cell Type	PWM
Footprinting	chr11:73698052..73698062	MyoD	Helas3lfna4h	
PWM	chr11:73698052..73698062	MyoD		

Figure 1b

Fig. 1. UCSC Genome Browser and RegulomeDB outputs for rs591758 at position 73,698,059 on chromosome 11 (based on dbSNP141 and hg19). **(a)** Genomic features around rs591758, where the vertical dotted line is located, are displayed by UCSC Genome Browser. The grey box in the DNase I Hypersensitivity Clusters track indicates a hypersensitive site to DNase I. The Transcription Factor ChIP-seq tracks show transcription factor binding sites from ChIP-seq experiments carried out by the ENCODE project. The DNA binding motifs are from the ENCODE Factorbook repository, which can be viewed as a matrix of all ENCODE transcription factor ChIP-seq datasets. The darkness is proportional to the signal strength, and the green segment highlights the highest scoring-site motif, with the arrows in the green segment denoting the matching strand (5' to 3') of the motif. **(b)** According to RegulomeDB, SNP rs591758 scores 2b ("likely to affect binding") for its potential effects on transcription factor binding, a motif, DNase footprint, and DNase peak. The SNP falls in FOXA protein binding sites (HepG2 is a hepatocellular carcinoma cell line). **(c)** The SNP also overlaps the

MyoD binding motif CANNTG. PWM is the position-weight matrix method for transcription factor binding. The base position in the motif that overlaps the SNP is shown in a box. HeLaS3lfna4h is a cervical carcinoma cell line.

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b

Figure 2a

Method	Location	Motif	Cell Type	PWM
Footprinting	chr11:73700391..73700411	CTCF	Mcf7Hypoxlac	
Footprinting	chr11:73700391..73700411	CTCF	Mcf7Hypoxlac	
Footprinting	chr11:73700391..73700411	CTCF	Mcf7	
Footprinting	chr11:73700391..73700411	CTCF	H1hesc	
Footprinting	chr11:73700392..73700411	CTCF	Mcf7Hypoxlac	
Footprinting	chr11:73700392..73700411	CTCF	Mcf7	

Figure 2b

Fig. 2. UCSC Genome Browser and RegulomeDB outputs for rs675547 at position 73,700,409 on chromosome 11 (based on dbSNP141 and hg19). **(a)** Genomic features around rs675547, where the vertical line is located, are displayed by the UCSC Genome Browser. The grey box in the DNase I Hypersensitivity Clusters track indicates a hypersensitive site to DNase I. The Transcription Factor ChIP-seq track shows a CTCF binding site from ChIP-seq experiments carried out by the ENCODE project. The DNA binding motif is from the ENCODE Factorbook repository, which can be viewed as a matrix of all ENCODE transcription factor ChIP-seq datasets. The darkness is proportional to the signal strength,

and the green segment highlights the highest scoring-site motif. Note that rs675547 is only 15 bp away from rs67870053, which is a 7-bp indel variation (-/ACCAACA from 73,700,388 to 73,700,394; MAF=0.46 by the 1000 Genomes Project). The last four base pairs of rs67870053 overlap the first four base pairs of the CTCF binding motif. **(b)** According to RegulomeDB, SNP rs675547 scores 2a (“likely to affect binding”) for its potential effects on transcription factor binding, matched TF motif, matched DNase footprint, and DNase peak. PWM is the position-weight matrix method for transcription factor binding. The SNP position adjoining the motif is shown in a box. Mcf7 is a mammary gland, adenocarcinoma cell line, and H1hesc is an embryonic stem cell line.

Fig. 3. SNPs in LD in the *UCP2/3* region on chromosome 11. The SNPs examined in this study are enclosed in red, dotted lines. The Green line represents *UCP2* and the blue *UCP3*, with SNP locations indicated. The LD plot was created using Haploview with the standard color scheme: bright red for $D' = 1$, $\text{LOD} \geq 2$ and all other colors for $D' < 1$ or $\text{LOD} < 2$ or both. The numbers shown inside the boxes are D' values multiplied by 100 and empty boxes represent the D' value of 1.

Fig. 4.

Genomic features around rs1626521, where the vertical line is located, are shown by the UCSC Genome Browser. SNP names enclosed in red, dotted lines are those shown in LD Block 2 in Fig. 3. SNPs whose names are colored black are in introns, green are in coding (synonymous), red in coding (non-synonymous), and blue in untranslated regions. At the top, below the coordinate scale, two splice variants are shown, and rs1626521 falls in the 3' untranslated region of the shorter transcript. Different colors in the histone modification tracks represent results from different cell lines, and peak levels show enrichment levels of the corresponding histone marks as determined by ChIP-seq assays of the ENCODE project. The grey boxes in the DNase I Hypersensitivity Clusters track indicate hypersensitive sites to DNase I with the darkness proportional to the sensitivity. The number to the left of each box shows the number of cell lines in which the region was hypersensitive. The Transcription Factor ChIP-seq tracks show transcription factor binding sites from ChIP-seq experiments carried out by the ENCODE project. The darkness is proportional to the signal strength, and the green highlights indicate the highest scoring-site motifs. The TargetScan Regulatory Sites track shows conserved microRNA target sites for conserved microRNA families in the 3' UTR regions of genes. Seven miRNA target sites were included in the track, but shown are the top three sites with highest performance prediction scores.

Fig. 5. Interaction plot for RMR and rs1626521 in prediction of FI₃₄ in female nonagenarians (N = 37) based on Table 4. There are only three individuals homozygous for the minor allele A (Table 1); therefore, these homozygotes (closed red circles each with an asterisk) were plotted together with heterozygotes as carriers of A (closed red circles; A/A or A/G) compared to non-carriers (open blue circles; G/G).

Table 1

Genotype frequencies of SNPs analyzed

SNP	A/A	A/B	B/B	MAF	HWE
rs1626521	20 (0.54)	14 (0.38)	3 (0.08)	0.27	> 0.05
rs591758	15 (0.41)	16 (0.43)	6 (0.16)	0.38	> 0.05
rs675547	13 (0.35)	16 (0.43)	8 (0.22)	0.43	> 0.05

MAF = minor allele frequency; HWE = Hardy-Weinberg equilibrium. The major and minor alleles (A and B, respectively) correspond to G/A for rs1626521, C/G for rs591758, and A/G for rs675547. The numbers in parentheses are the proportions of the respective genotype in the sample

Table 2
Basic characteristics of the LHAS nonagenarian subjects (mean $\pm$ standard deviation)

Variable	Male (n=30)	Female (n=37)	All (n=67)
Age (years)	92 $\pm$ 2	92 $\pm$ 2	92 $\pm$ 2
FI ₃₄ **	0.183 $\pm$ 0.073	0.232 $\pm$ 0.069	0.210 $\pm$ 0.075
EESI (kcal/w)	3731.5 $\pm$ 3038.3	3632.8 $\pm$ 2862.8	3677.7 $\pm$ 2921.3
RMR (kcal/d) ***	1281.8 $\pm$ 147.3	1029.2 $\pm$ 122.0	1142.3 $\pm$ 183.5
FFM (kg) ***	52.1 $\pm$ 5.1	37.4 $\pm$ 5.3	44.0 $\pm$ 9.5

EESI = Energy Expenditure Summary Index of the Yale Physical Activity Survey; RMR = resting metabolic rate; FFM = fat-free mass;

**
 p 0.01 and

 p 0.001 by Wilcoxon test between female and male

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Table 3

Association of *UCP2* variants with FI_{34} in female nonagenarians

	rs591758		rs675547		rs1626521	
	b	se	b	se	b	se
RMR	2.7e-4 **	7.7e-5	2.8e-4 **	8.5e-5	2.3e-4	8.6e-5
SNP	4.3e-2 **	1.3e-2	3.1e-2 *	1.4e-2	-8.1e-3	1.6e-2
R ²	0.34		0.24		0.13	
P	0.00035		0.0035		0.032	

b = regression coefficient

* *p* 0.05 and

** *p* 0.01);

se = standard error of b; RMR = resting metabolic rate; SNP = additive genetic model for each SNP; R²= adjusted R²; *P* = *p* value of the coefficient of determination (R²) obtained by application of the F-statistic in analysis of variance; sample size (n = 37)

Table 4
Interaction between RMR and the *UCP3* variant explains *FI*₃₄ in female nonagenarians

	rs591758		rs675547		rs1626521	
	b	se	b	se	b	se
RMR	2.4e-4	1.4e-4	1.4e-4	1.6e-4	-7.1e-5	1.5e-4
SNP	1.6e-2	0.11	-9.4e-2	0.12	-0.40 *	0.16
RMRxSNP	2.6e-5	1.1e-4	1.2e-4	1.2e-4	3.9e-4 *	1.6e-4
R ²	0.32		0.24		0.25	
P	0.0013		0.0066		0.0057	

b = regression coefficient

* *p* 0.05 and
*** *p* 0.01);

se = standard error of b; RMR = resting metabolic rate; SNP = additive genetic model for each SNP; R²_{adj} = adjusted R²; *P* = *p* value of the coefficient of determination (R²) obtained by application of the F-statistic in analysis of variance; sample size (n = 37)

Table 5
Association of *UCP2/3* SNPs with FI_{34} in female nonagenarians after adjustment for muscle mass and physical activity

	rs591758		rs675547		rs1626521	
	b	se	b	se	b	se
FFM	-6.0e-3 **	2.1e-3	-6.3e-3 **	2.3e-3	-5.1e-3 *	2.5e-3
EESI	-8.6e-6 **	2.8e-6	-9.1e-6 **	3.0e-6	-1.1e-5 **	3.3e-6
RMR	4.4e-4 ***	9.2e-5	4.6e-4 ***	1.0e-4	3.7e-4 **	1.1e-4
SNP	4.0e-2 **	1.1e-2	3.1e-2 *	1.2e-2	1.2e-3	1.6e-2
R ²	0.56		0.49		0.38	
P	< 0.0001		< 0.0001		0.00066	

b = regression coefficient

* p 0.05,

** p 0.01,

*** p 0.001);

se = standard error of b; FFM = fat-free mass; EESI = energy expenditure summary index in the Yale Physical Activity Survey; RMR = resting metabolic rate; SNP = additive genetic model for each SNP;
R² = adjusted R²; P = p value of the coefficient of determination (R²) obtained by application of the F-statistic in analysis of variance; sample size (n = 37)

Interaction between RMR and the *UCP3* variant in female nonagenarians explains FI_{34} after adjustment for muscle mass and physical activity

Table 6

	rs591758		rs675547		rs1626521	
	b	se	b	se	b	se
FFM	-5.7e-3 **	2.1e-3	-5.4e-3 *	2.3e-3	-3.6e-3	2.3e-3
EESI	-9.7e-6 **	3.0e-6	-1.1e-5 **	3.1e-6	-1.2e-3 ***	2.9e-6
RMR	3.1e-4 *	1.5e-4	2.1e-4	1.7e-4	5.1e-6	1.5e-4
SNP	-6.5e-2	9.7e-2	-0.15	0.11	-0.41 **	0.13
RMRxSNP	1.0e-4	9.5e-5	1.8e-4	1.0e-4	4.1e-4 **	1.3e-4
R^2	0.56		0.52		0.52	
P	< 0.0001		< 0.0001		< 0.0001	

b = regression coefficient

* p 0.05,

** p 0.01,

*** p 0.001);

se = standard error of b; FFM = fat-free mass; EESI = energy expenditure summary index in the Yale Physical Activity Survey; RMR = resting metabolic rate; SNP = additive genetic model for each SNP; R^2 = adjusted R^2 ; P = p value of the coefficient of determination (R^2) obtained by application of the F-statistic in analysis of variance; sample size (n = 37)