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UCP3 polymorphisms, hand grip performance and survival at old age: Association analysis in two Danish middle aged and elderly cohorts

Serena Dato [a,b,*], Mette Soerensen [b,c,d], Alberto Montesanto [a], Vincenzo Lagani [e], Giuseppe Passarino [a], Kaare Christensen [b,c,d], Lene Christiansen [b,c,d]

[a] Department of Cell Biology, University of Calabria, Ponte Pietro Bucci cubo 4C, 87036 Rende (CS), Italy
[b] The Danish Aging Research Center, Epidemiology, Institute of Public Health, University of Southern Denmark, J.B. Winsloews Vej 9B, 5000 Odense C, Denmark
[c] Department of Clinical Genetics, Odense University Hospital, Sdr. Boulevard 29, 5000 Odense C, Denmark
[d] Department of Clinical Biochemistry and Pharmacology, Odense University Hospital, Sdr. Boulevard 29, 5000 Odense C, Denmark
[e] Bio Informatics Laboratory, Institute of Computer Science, Foundation for Research and Technology (Hellas), Heraklion, Greece

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ABSTRACT

An efficient uncoupling process is generally considered to have a protective effect on the aging muscle by slowing down its age-related decay. Genetic polymorphisms in the Uncoupling Protein 3 (*UCP3*) gene, whose product is mainly expressed in skeletal muscle, were suggested to be associated with hand grip (HG) performances in elderly populations. Considering the population specificity of the quality of aging, we aimed to add further support to this evidence by analyzing the association between four SNPs in the *UCP3* gene and relative haplotypes in two large cohorts of middle aged ($N = 708$) and oldest old Danes ($N = 908$). We found that the variability at rs1685354 and rs11235972 was associated with HG levels both at single and haplotypic level in both cohorts. Furthermore, taking advantage of large cohort and period survival data of the oldest cohort, we tested the association of each SNP with survival at 10 years from the baseline visit. Interestingly, we found that allele A at rs11235972, associated in this cohort with lowest HG scores, influences also the survival patterns, with people carrying this allele showing higher mortality rates. On the whole, our work supports the role of *UCP3* gene in functional status and survival at old age.

1. Introduction

The reduction of muscle mass in the elderly, known as sarcopenia, becomes so significant after the fifth decade of life that it is widely studied as one of the most important markers of human aging. Furthermore, sarcopenia is regarded as one of the major contributors to frailty (Morley et al., 2001), considering that the loss of muscle mass influences metabolic adaptation, immunological response to disease, capability to respond to environmental stress (Schrager et al., 2003) and so has a relevant impact on the overall homeostasis at old age. Operatively, sarcopenia is measured through the reduction of hand grip strength, which is considered a measure of the overall skeletal muscle mass, observed to gradually and significantly decrease as the individual ages (Doherty, 2003). Hand grip strength is generally considered a very reliable marker of the functional status, as well as one of the most effective predictors of disability and mortality in the elderly (Rantanen et al., 1999; Metter et al., 2002; Nybo et al., 2003; Newman et al., 2006). Furthermore, twin studies demonstrated that about 50% of the observed variation in hand grip strength can be explained by genetic effects (Frederiksen et al., 2002), thus pointing out this phenotype as a useful marker for the identification of genes affecting mid- and late-life physical functioning. Several associations have been reported between hand grip and the variability of genes involved in inflammatory response, myostatin signaling, neuron and bone metabolism (Roth et al., 2001; Arking et al., 2006; De Mars et al., 2007; Walsh et al., 2009; Dato et al., 2010; Windelinckx et al., 2011). Recently the Uncoupling Protein (*UCP*) genes have been put forward as candidate genes, because of their role in energy metabolism. Of particular interest is the Uncoupling Protein 3 (*UCP3*) gene, since this is expressed in skeletal muscle where it regulates fatty acid metabolism, oxidative status, and Reactive Oxygen Species (ROS) production. A previous report showed that a functional polymorphism, located in the promoter region of the *UCP3* gene (rs1800849), significantly affects hand grip strength in an elderly population from southern Italy (Crocco et al., 2011), suggesting a correlation between the uncoupling process and the regulation of muscle metabolism/catabolism in the elderly.

* Corresponding author at: Department of Cell Biology, University of Calabria, 87036 Rende, Italy. Tel.: +39 0984 492933; fax: +39 0984 492911.
E-mail address: s.dato@unical.it (S. Dato).

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In addition, recent evidences report as the genetic variability of *UCP2*, *UCP3* and *UCP4* affects the individual's chances of surviving up to a very old age in the same population, thus confirming the relevance of the uncoupling pathway as candidate for human longevity (Rose et al., 2011a, 2011b).

In this study we aimed to add further support to the association of *UCP3* variability with hand grip strength, by investigating four Single Nucleotide Polymorphisms (SNPs) and their relevant haplotypes in a different population, composed by two large cohorts of middle aged and oldest old Danes, well characterized by a phenotypic point of view. Moreover, taking advantage of the longitudinal study design of the oldest cohort, we investigated the association of *UCP3* variations with survival at old ages.

2. Materials and methods

2.1. Samples

Two Danish samples were analyzed, for a total of 1616 (621 men and 995 women) subjects. The oldest old sample included 908 subjects 93 years old (265 males and 643 females), drawn from the Danish 1905 Cohort, a population-based nationwide survey of all Danish people born in Denmark in 1905 (Nybo et al., 2001, 2003; Christensen et al., 2008). Briefly, 2262 people were initially recruited in 1998, when they were 92–93 years of age. Vital status was ascertained every year, from the first visit to January 1st 2010 or until death whichever came first, resulting in a mean follow-up time for survivors of 11.4 years (range: 11.2–11.6). Information on death for all the cohort members was retrieved from the Danish Central Population Register, which since 1968 keeps a record of all those living in Denmark and which is continuously updated (Pedersen et al., 2006). The participation rate of the survey was 63% and comparisons of demographical characteristics of participants with non participants demonstrated that recruited people were a fairly non-selected group of the 1905 Cohort (Nybo et al., 2001).

The younger sample (mean age 50.5) included 708 subjects (356 males and 352 females) randomly selected from the Study of Middle-Aged Danish Twins (MADT) (Gaist et al., 2000). Recruitment was started in 1998, when 2640 intact twin pairs from 22 consecutive birth years (1931–1952) were chosen via the Danish Central Population Register. The sample analyzed in this study includes only one twin from each twin pair.

Both surveys included multidimensional face-to-face interviews, aimed at the collection of socio-demographic information, assessment of physical, cognitive, depressive status, sensory impairments, medications, self-reported health status and DNA sampling. Permission to collect blood samples and usage of register based information was granted by The Danish National Committee on Biomedical Research Ethics.

2.2. Measurement of hand grip strength

Hand grip (HG) strength was measured by a handheld dynamometer (SMEDLEY's dynamometer TTM, Tokyo, Japan) while the subject was sitting with the arm close to his/her body. The test was repeated three times with the stronger hand; the maximum of these values was used in the analyses. When a test was not carried out, it was specified whether it was due to physical disabilities or because the subject refused to participate.

2.3. SNP selection and genotyping

For the analysis of *UCP3* genetic variability, SNPs were chosen in the genomic region 73,387,985–73,402,778 of chromosome 11, which corresponds to the coding region of *UCP3* gene plus 5000 base pairs (bp) upstream and 1000 bps down stream (NCBI B36 assembly), in order to take into consideration possible regulatory elements of the gene. Tagging SNPs covering as much as possible of the known common genetic variation in *UCP3* were chosen by analyzing HapMap genotype SNP data in *HaploView* software (http://www.broadinstitute.org/haploview/haploview, Barrett et al., 2005). In this analysis, the following criteria were adopted: Minimum Allele Frequency (MAF) of at least 5%, 'pair wise tagging only', $r^2 \geq 0.8$, LOD = 3 and a minimum distance between SNPs = 60 bp. The same program has been used for obtaining the graphical overview of Linkage Disequilibrium (LD) (definition of blocks based on confidence interval algorithm, as reported in Gabriel et al., 2002). Pairwise measures of LD between the analyzed loci were quantified by the correlation coefficient r^2.

DNA was isolated from blood spot samples using the QIAamp DNA Mini and Micro Kits (Qiagen). Genotyping of the 4 *UCP3* SNPs (rs11235972, rs1685354, rs3781907 and rs647126, general characteristics reported in Table 1SM, Supplementary material) was performed as described in Soerensen et al. (2012), by using the Illumina GoldenGate platform (Illumina Inc) and data cleaning was carried out according to Illumina Inc's recommendations.

2.4. Statistical analyses

For each *UCP3* polymorphism, allele frequencies were estimated by gene counting from the observed genotypes. Hardy–Weinberg equilibrium was tested by a Monte-Carlo approach based on 10,000 random allele permutations (Weir, 1996). Standard errors for alleles were computed according to the hypothesis of the multinomial distribution.

2.4.1. Single-locus analysis

In order to assess phenotype–genotype associations we used the F_{max} statistics proposed by Lettre et al., 2007. Briefly, this approach allows obtaining a robust association test for quantitative traits within a linear regression framework using the best F statistics (F_{max}) observed for different genetic models (additive, dominant and recessive) taking into account also possible covariates' effects.

In the present study, the F_{max} test has been applied to investigate the relationship between *UCP3* polymorphisms and the HG performances. The variables age at intake, gender and Body Mass Index (BMI) were used as adjunctive covariates in the regression analysis related to the MADT Cohort, while gender and BMI were included in the case of 1905 Cohort. A permutation approach (10,000 permutations) was used to evaluate the significance of the statistics obtained.

2.4.2. Haplotypic analysis

In order to model the effect of the *UCP3* haplotypes on the HG performances, we used a haplotype-based association analysis within the Generalized Linear Model (GLM) framework, that allows to handle ambiguous haplotypes. The *haplo.score* function of *haplo.stat* package in R has been used to obtain the GLM-based score statistics for testing both global and individual haplotype effects on the HG performance (Schaid et al., 2002). In this model the effects of the different haplotypes were assessed by assuming an additive model. As in the previous case, the variables age at intake, gender and BMI were used as adjunctive covariates in the MADT Cohort, while gender and BMI were included in the case of 1905 Cohort. A permutation approach has been used to evaluate the significance of the scores obtained.

2.4.3. Survival analysis

In order to evaluate if the detected effects of the analyzed *UCP3* polymorphisms on the HG performance might finally result in differential patterns of survival of the different relevant genotypes, we evaluated survival after 10 years from the baseline visit in the 1905 Cohort by using Accelerated Failure Time (AFT) models (Bradburn et al., 2003). Sex, BMI and the four *UCP3* SNPs (a SNP each time) were considered as covariates. As reported in the paper by Dato et al. (2011), AFT models can be preferred over the more commonly used Cox regression model (Cox, 1972), particularly when, as in the case of the variable "sex" for the 1905 Cohort, proportional hazard assumptions are not respected (Schoenfeld, 1982, see Supplementary materials). Moreover, with respect to the less intuitive hazard function provided by Cox models, AFT models allow obtaining a clearer explanation of covariate effects, by directly linking the estimation of survival time with the values of covariates: a positive regression coefficient indicates that the covariate is associated with higher survival chances; conversely for negative coefficients. In addition, recent study demonstrated the usefulness of AFT models also in aging research (Swindell, 2009). Then, from the fitted AFT models, sex-adjusted survival curves were obtained, analyzing each SNP independently from each other. Fitting of AFT models was carried out by assuming a Weibull distribution. Subjects alive or immigrated after 10 years from the baseline visit were considered as censored.

Survival analysis of UCP3 haplotypes was carried out by using AFT models as well. In particular, first the probabilities of the individual haplotype combinations (diplotypes) were estimated by using an Expectation Maximization (EM) algorithm implemented in the haplo.stats package of R. Second, N dummy variables, corresponding to the number of different observed haplotypes, were obtained coding the reconstructed diplotype in an additive fashion (the count of a particular haplotype in the diplotype). Finally, AFT models were used for estimating the statistical significance of each haplotype, with the EM – estimated probabilities used as weights. Since this approach underestimates the standard errors of rare haplotypes, they were not included in the model when their frequency was lower than 5%. As in the previous case, sex and BMI were used as adjunctive covariates in the model.

All statistical analyses were carried out using R statistical environment (R Development Core Team, 2010. R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria 2011, URL http://www.R-project.org). In particular, *survival* package was used for survival analyses; *haplo.stats and Design* packages for haplotypes association analyses (Sinnwell and Schaid, 2009; Harrell and Frank, 2009). A significance level of 0.05 was set for all the tests.

3. Results

Table 1 shows the distribution of the observed genotypes and allele frequencies of *UCP3* SNPs in the MADT and 1905 Cohorts. For each SNP no significant departure from Hardy–Weinberg equilibrium was observed in both samples.