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Contribution of genetic polymorphisms on functional status at very old age: A gene-based analysis of 38 genes (311 SNPs) in the oxidative stress pathway

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Abstract

Preservation of functional ability is a well-recognized marker of longevity. At a molecular level, a major determinant of the physiological decline occurring with aging is the imbalance between production and accumulation of oxidative damage to macromolecules, together with a decreased efficiency of stress response to avoid or repair such damage. In this paper we investigated the association of 38 genes (311 SNPs) belonging to the pro-antioxidant pathways with physical and cognitive performances, by analyzing single SNP and gene-based associations with Hand Grip strength (HG), Activities of Daily Living (ADL), Walking Speed (WS), Mini Mental State Examination (MMSE) and Composite Cognitive Score (CCS) in a Cohort of 1089 Danish nonagenarians. Moreover, for each gene analyzed in the pro-antioxidant pathway, we tested the influence on longitudinal survival.

In the whole sample, nominal associations were found for *TXNRD1* variability with ADL and WS, *NDUFS1* and *UCP3* with HG and WS, *GCLC* and *UCP2* with WS ($p < 0.05$). Stronger associations although not holding the multiple comparison correction, were observed between

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MMSE and *NDUFV1*, *MT1A* and *GSTP1* variability ($p < 0.009$). Moreover, we found that association between genetic variability in the pro-antioxidant pathway and functional status at old age is influenced by sex. In particular, most significant associations were observed in nonagenarian females, between HG scores and *GLRX* and *UCP3* variability, between ADL levels and *TXNRD1*, MMSE and *MT1A* genetic variability. In males, a borderline statistically significant association with ADL level was found for *UQCERS1* gene.

Nominally significant associations in relation to survival were found in the female sample only with *SOD2*, *NDUFS1*, *UCP3* and *TXNRD1* variability, the latter two confirming previous observations reported in the same cohort. Overall, our work supports the evidence that genes belonging to the pro-anti-oxidant pathway are able to modulate physical and cognitive performance after the ninth decade of life, finally influencing extreme survival.

Keywords

Oxidative stress; 1905 Danish Cohort; Aging; Survival at old age; Functional status

1. Introduction

Aging is a very complex phenomenon, which negatively impacts the functioning of different biological systems, leading to a systemic impairment and ultimately to death. The physiological decline occurring with aging can be determined by an imbalance between the production and accumulation of oxidative damages to macromolecules, and cellular ability to repair such damage (Balaban et al., 2005; Harman, 2009). The balance between pro-oxidants and enzymatic anti-oxidant systems may be of particular importance in the elderly, whose nutritional deficiencies and sedentary lifestyle concur with a depletion of dietary antioxidants and increased susceptibility to oxidative stress.

Decline of functional capacity and stress resistance to internal and external factors are strictly related (Simm et al., 2008) and together can be used as measures of the aging rate (Vasto et al., 2010).

The importance of the oxidative stress pathway in aging and longevity, in particular, was firstly proposed by Harman in 1956 with the “oxidative stress theory of aging” (Harman, 1956) and supported by genetic screenings in model organisms, demonstrating that longevity can be promoted through the manipulation of metabolism (i.e. caloric restriction) and resistance to oxidative stress (Vijg and Suh, 2005). Despite considerable divergence during evolution, the same mechanisms appear to act in mammals too and it is considered that decreased metabolism and increased resistance to oxidative stress represent key mechanisms of prolonged lifespan in mutant mice (Bartke et al., 2001). In humans, the worsened efficiency of stress response represents a health risk, leading to the onset and accrual of major age-related diseases, including Alzheimer’s, cancer, cardiovascular diseases (CVD) and diabetes (Lloret et al., 2008). Moreover, an association between oxidative imbalance and frailty syndrome was demonstrated, with an increase of oxidative stress in frail, institutionalized elderly people, leading to an acceleration of aging processes in comparison with free living elderly (Serviddio et al., 2009).

Although many association studies explored the effect of genetic variability at candidate genes belonging to the oxidative stress pathway in relation to age-related clinical conditions (Crawford et al., 2012), as well as with human longevity (see Dato et al., 2013 for a complete list), few papers investigated their role on the quality of human aging, and in particular on the functional decline characterizing human senescence. To date, efforts were mainly devoted to investigating the association of genes related to oxidative stress with cognitive ability and cognitive aging in healthy older people (Deary et al., 2004; Harris and Deary, 2011; Harris et al., 2007; Kachiwala et al., 2005; Starr and Quinn, 2008), mainly because brain is particularly vulnerable to oxidative damage as a result of its high aerobic metabolism and high concentration of polyunsaturated fatty acids, which are susceptible to lipid peroxidation (Halliwell, 2006). These studies found an involvement of apolipoprotein E (*APOE*), catechol-O-methyl transferase (*COMT*), brain-derived neurotrophic factor (*BDNF*) and dystrobrevin binding protein 1 (*DTNBPI*) genes in cognitive ability in older people; however, they did not consider very old subjects, analyzing sample population aged around 85 years as maximum (De Blasi et al., 2009; Lindenberger et al., 2008).

Preservation of functional ability (together with cognitive performance) is a well-recognized marker of longevity (Schupf et al., 2004). Consistently, predictors of functional status as HG and disability are key components of frailty definition at old age (Dato et al., 2012a; Montesanto et al., 2010).

Furthermore, analyses of longitudinal cohorts of Danish nonagenarians demonstrated that exceptional longevity does not result in excessive levels of disability (Christensen et al., 2008), suggesting a relationship between the general increase of life expectancy with a good maintenance of individual activities of daily living. This evidence supports the hypothesis that factors influencing the increased survival can also influence disability levels in older cohorts (Engberg et al., 2008) and so that longevity and lower disability levels may have common causes at organismal level.

In this study, we investigated the association between 311 single nucleotide polymorphisms (SNPs) at 38 genes belonging to the oxidative stress pathway with functional status at very old age through a gene-based approach. To this purpose, we analyzed 1089 Danish nonagenarians, drawn from the longitudinal 1905 Cohort Study, a well characterized (Christensen et al., 2009; Nybo et al., 2001, 2003) and very suitable sample for genotype–phenotype association studies (Dato et al., 2010; Soerensen et al., 2010, 2012a,b, 2013) for the associations with physical and cognitive parameters, respectively represented by HG strength, ADL, WS, MMSE and CCS.

Moreover, for verifying a possible effect of each gene on mortality, we tested the association with survival after the ninth decade of age.

2. Materials and methods

2.1. Samples

1089 Danish samples were analyzed (313 males and 776 females), belonging to the “Danish 1905 Cohort Study”. General characteristic of this population and post-survey mortality data are described in Table 1.

Briefly, this population based survey started in 1998, when 2.262 of the participants were 92.22–93.82 years of age (mean age 93.2), and survivors were subsequently re-assessed in 2000, 2003 and 2005 (Christensen et al., 2008; Nybo et al., 2001, 2003). Home-based questionnaires were submitted for the collection of socio-demographic information, assessment of physical, cognitive, depressive status, sensory impairments, medications, self-reported health status and DNA was sampled for each subjects participating to the survey. Vital status was followed until death or January 1st 2013, whichever came first, resulting in a mean follow-up time for survivors of 13.4 years (range: 13.2–13.6). Information on survival status was retrieved from the Danish Central Population Register (Pedersen et al., 2006).

Permission to collect blood samples and usage of register-based information was granted by The Danish National Committee on Biomedical Research Ethics, both for initial and follow-up study.

2.2. Genetic data

Genetic data analyzed in this work were collected in the frame of a project on the study of genetic determinants of human longevity, described in Soerensen et al. (2012b), where 148 genes (1273 SNPs) belonging to three candidate pathways (GH/IGF-1/Insulin signaling, DNA damage signaling and repair and pro–anti-oxidant pathways) were investigated in two cohorts of middle aged and oldest old Danes. Briefly, candidate genes were chosen by integrating a comprehensive analysis of literature and database search. SNP selection was carried out through a dedicated protocol, which takes into account both candidate SNPs for aging and age-related diseases in each analyzed pathway and coding or potentially functional SNPs (non-synonymous SNPs, SNPs located in potential splice sites or transcription factor binding sites and SNPs potentially inducing frame shifts or nonsense-mediated mRNA decay). Finally, HAPMAP tagging SNPs were added for reasonably covering the genetic variability in the gene region. Supplementary Table 1S reports the complete list of the 311 SNPs relative to 38 genes belonging to the pro–anti-oxidant pathway analyzed in this study.

2.3. Functional parameters and survival outcome

2.3.1. Disability—The management of activities of daily living or ADL (toileting, getting up from bed, raising from a chair, walking around) was assessed by using a modification of an international and widely used scale, the Katz’ Index of activities of daily living (ADL) (Katz et al., 1970). The assessment was based on what the subject was able to do at the time of the visit. Each activity was scored as 0 when the subject was unable to perform the

activity analyzed and 1 when able to perform such activity. In the following analysis, ADL scores were dichotomized as 1 if the subject was independent in all items, and 0 otherwise.

2.3.2. Physical performance—Physical performance was assessed by evaluating HG strength and WS performances. HG 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 and the maximum of these values was used in the analyses. When a test was not carried out, it was specified if it was due to physical disabilities or because the subject refused to participate. Walking time was measured as the best performance (shortest time in seconds) of two walks along a defined distance. Since two different walking distances (3 or 4 m) were measured, in order to make these two performances comparable we adopted the participant's usual gait-speed (in m/s) as a measure of performance, defined in this paper as WS.

2.3.3. Cognitive functioning—Screening of cognitive impairment was carried out by MMSE and Composite Cognitive Score tests. MMSE is a 30-point cognitive scale which evaluates several different areas of thinking including memory, judgment, calculation, abstraction, language, and visual-spatial ability (Folstein et al., 1975). MMSE scores range from 0 (lowest cognitive function) to 30 (highest cognitive function). Since the test is affected by age and educational status, the MMSE scores were normalized for these variables.

Composite cognitive score is computed by aggregating five single scores: a fluency test, forward digit span, backward digit span, immediate recall and delayed recall of a 12-item list. Single items were individually standardized to a mean of 0 and standard deviation of 1, as suggested by McGue and Christensen (2001), for facilitating the interpretation of results.

2.3.4. Survival outcome—The time to event was defined as the time elapsed from the visit until death. The average time to event is 1290 days. The data were not affected by censoring, i.e., all the subjects included in the analysis after the quality controls were dead at the time of the analyses.

2.4. Statistical analyses

2.4.1. Quality-control—After genotype calling, in order to eliminate possible bias on the analysis due to a low calling in subject and/or SNP data, the dataset was subjected to a battery of quality-control (QC) tests. On the subject level, samples were excluded if they had a call fraction lower than 90%. On the genotype level, SNPs were excluded if they had (1) a missing frequency (MiF) higher than 10% and (2) a frequency of rare allele (MAF) < 1%.

2.4.2. Single-SNP analysis—The association between the analyzed genetic variants and the quantitative/dichotomous traits was assessed by using linear/logistic regression models. ANOVA and the likelihood ratio tests, as appropriate, were adopted to assess the significance of the resulting associations. Since for each SNP three different coding schemes were adopted (dominant, recessive and additive), the significance of the association was summarized with the MAX statistic approach used in Li et al. (2008). A complete

description of this procedure is reported in the Supplementary material (see Statistical Appendix).

The association between genetic variants and survival time was carried out with the same approach employed for the quantitative and dichotomous traits. Cox regression models were used in place of linear and logistic models, in order to better capture the idiosyncrasies of the survival outcome (see Statistical Appendix).

2.4.3. Gene-based analysis—For each analyzed gene, the gene-based analysis was carried out using the following algorithm:

1. perform single SNP association analysis as describe before;
2. compute the gene-based statistic as the average of these single SNP statistics;
3. permute the dataset 10,000 of times, keeping LD between SNPs constant (i.e. permute phenotype labels);
4. for each permuted dataset, repeat steps 1 to 2 and build the null-distribution;
5. 5. obtain the empirical p-value for the analyzed gene by comparing the original statistics with the permutation-based null-distribution.

For the genes with at least one SNP showing a significant sex-specific effect ($p < 0.05$), gene-based analyses were also carried out by sex.

The empirical p-values obtained in the gene-based analysis have been adjusted for multiple comparisons using the Bonferroni correction.

For all calculations, the *R* statistical environment was used (R Development Core Team, 2011; <http://www.r-project.com>).

3. Results

3.1. QC analysis

After the QC phase, the final number of samples was 1080 (311 males and 769 females) including high quality genotypes for a total of 293 SNPs. In particular, 9 individuals were excluded from the analysis because of a call fraction lower than 90%. One SNP was excluded from the analysis because of a MiF value lower than 90% and one on the basis of MAF criterion ($<1\%$) (see Supplementary Table 1S for details).

3.2. Gene-based analysis

Gene-based association analysis was carried out among the 38 genes in the pro–anti-oxidant pathway and the five parameters of functional status (ADL, HG, WS, MMSE, CCS) plus survival. Table 2 lists the 14 genes showing a nominally significant association with at least one trait under study in the whole sample ($p < 0.05$). For each of them, the SNP showing the higher contribution to the association has been reported as best SNP.

Association with ADL performance was found for the *TXNRD1* gene only ($p = 0.010$), which reported 6 nominally significant associations ($p < 0.05$) with this phenotype (see Supplementary Table 2S_1) at single SNP level.

As it regards the HG performance, two nominally significant associations were detected. The former, in line with previously published data (Dato et al., 2012b), involved the *UCP3* gene ($p = 0.019$) with the best SNP rs11235972 confirming the association with less HG strength previously reported in the 1905 cohort; the latter involved *NDUFS1* gene ($p = 0.022$). In the case of WS performance, the strongest association was detected for *GCLC* gene ($p = 0.017$). An effect on WS was found also for *UCP2* ($p = 0.032$), *UCP3* ($p = 0.027$) and *NDUFS1* ($p = 0.025$) too, the last two confirming the association with physical status suggested by the correlation with HG scores; a borderline association with WS performance was detected for *TXNRD1* gene too ($p = 0.053$), that was also associated with ADL levels in this cohort.

As for MMSE, three strong associations were found, related to the *NDUFV1* gene ($p = 0.005$), the *MTIA* gene ($p = 0.006$) and the *GSTP1* gene ($p = 0.009$), all having a favorable effect on cognitive functioning at single SNP level. Less significant but still under the nominal level were the associations with MMSE levels of *UQCERS1* gene ($p = 0.038$) and *GLRX* ($p = 0.041$). Borderline significant was the association between *PRDX3* gene and cognitive functioning ($p = 0.049$). No one of these genes was found associated with the performance at CCS, which showed a borderline association with *ACOX1* genetic variability only ($p = 0.048$).

Finally, no gene found associated with a functional phenotype showed a corresponding association with survival. However, two nominally significant associations with survival have been found in the whole sample, in relation to *LOX* ($p = 0.013$), *SOD2* ($p = 0.012$) and *UQCERS1* ($p = 0.038$) genes, which variability were found to positively (*LOX*) and negatively (*SOD2*, *UQCERS1*) influence the survival at gene level in this Cohort. *TXNRD1*, although showing a significant association with survival at SNP level (see Supplementary Table 2S_2), was not found associated with survival at gene level in the whole sample.

After adjusting for multiple comparisons, none of the associations reported in the whole sample studied hold the statistical significance (Bonferroni corrected threshold: $p < 1.3 \times 10^{-3}$).

Gene-based analysis stratified by sex was carried out for genes carrying at least one SNP showing a sex specific effect (p -value < 0.05 in Tables 2S_1 and 2S_2) on the analyzed traits in single SNP analysis. Table 3 reports the genes showing significant association at a nominal level of 0.05 with at least one trait under study plus survival in nonagenarian males (a) and females (b).

In males, the most interesting result was observed for *UQCERS1* gene with ADL performance ($p = 0.002$) and *UCP2* with WS ($p = 0.009$), the first one borderline significant also after the adjustment for multiple comparisons. Other nominally significant associations were found for *SOD3* ($p = 0.031$) and *XDH* ($p = 0.020$) with ADL, *GCLC* ($p = 0.024$) with HG, *CP* ($p = 0.021$) and *CYC1* ($p = 0.022$) with CCS. Borderline significant to a nominal

level were the association of *GPX4* ($p = 0.045$) and *NOS3* ($p = 0.052$) with ADL and MMSE, respectively. No association with survival was reported in males for genes belonging to the pro-oxidant pathway here analyzed.

In females, a higher number of nominally significant associations were reported. The one of *GLRX* with HG levels ($p = 0.001$) hold also the correction for multiple comparisons ($p < 1.3 \times 10^{-3}$). Borderline significant also after correction for multiple testing ($p = 0.002$) are the associations of *TXNRD1* gene with ADL scores, *MT1A* gene with MMSE performances and *UCP3* with HG, in line with those found in the whole sample and reflecting the higher number of females in this Cohort. Other notable associations were observed for *TXN2* with ADL ($p = 0.038$), *UCP2* with HG strength ($p = 0.008$), *SOD2* ($p = 0.005$) and *GCLC* ($p = 0.007$) with WS performances.

As for survival, four nominally significant association with lifespan were found in the female cohort, concerning the genes *NDUFS1* ($p = 0.009$), *TXNRD1* ($p = 0.023$), *SOD2* ($p = 0.035$) and *UCP3* ($p = 0.041$). This result is consistent with their associations with physical functioning and suggests that the variability of genes in the pro-antioxidant pathway can influence survival through an effect on physical performances, at least in the analyzed cohort.

4. Discussions

The maintenance of a good functional ability (together with cognitive performances) is a well-recognized marker of longevity (Schupf et al., 2004). Physical activity has many well-established health benefits, going from the maintenance of muscle mass to the delay of cognitive impairment, to the promotion of a psychological wellness; however, it is known that a strenuous exercise can be disadvantageous for longevity, because it can increase muscle oxygen flux, stimulating intracellular events leading to an enhanced oxidative stress (Dato et al., 2013 and references therein). A moderate exercise can instead induce anti-oxidant adaptation, thereby balancing oxidative stress and muscle deterioration, as demonstrated by an increase of two years in the average lifespan and a delayed onset of disabilities of physically active people in comparison with less active (Ji, 2002). Progressive oxidative damage is also a conserved central mechanism of age-related cognitive decline, because of the higher consumption of oxygen (more than 20% than other tissues) in the brain, with the consequent exposition to ROS from mitochondrial respiration. As for the muscle mass, a regular physical activity seems to protect against brain damage, by preventing brain aging and neurodegeneration (Cotman and Engesser-Cesar, 2002; Dik et al., 2003).

Thus, physically active people should benefit from exercise-induced adaptation in cellular antioxidant defense systems, becoming less vulnerable to acute injury and chronic inflammation, finally delaying the onset of chronic illness and improve their quality of life (Dato et al., 2013).

In this work, to the aim of investigating the possible influence of the pro-anti-oxidant pathway to functional status at old age, we tested the association of genes belonging to pro- and anti-oxidant pathways with predictors of physical (HG, ADL and WS) and cognitive

decline (MMSE and CCS) at old age, in a cohort of Danish nonagenarians. Few studies investigated the genetic variations influencing physical abilities at old age and samples were mainly drawn from hospitalized and affected patients. Positive association was reported between ADL levels and *ACE* gene by Seripa et al. (2011), while no correlation was found with *APOE* gene by Kulminski et al. (2008) and Bader et al. (1998), the last in a cohort of 253 healthy and disabled Italian octo- and nonagenarians. As for cognitive functioning, studies found an involvement of genes specifically involved in neurological metabolism and dopaminergic neuromodulation, as *APOE*, *COMT*, *BDNF* and *DTNBP1* genes, with cognitive ability in older adults less than 85 years (De Blasi et al., 2009; Lindenberger et al., 2008). In particular, studies demonstrated an involvement of genetic variability on specific cognitive areas; moreover, they suggest that aging magnifies the functional consequences of common genetic polymorphisms on specific abilities, such as executive functioning and working memory, contributing to the marked heterogeneity in late-life cognitive functioning.

We moved forward exploring the association with functional status of genes belonging to a specific metabolic pathway, demonstrated to be biologically involved in physical and cognitive impairment.

Overall, only 2 of the top 14 genes found associated with functional status in this work have been previously reported in association studies with physical status at old age, in Italian and Danish Cohorts (the latter is the same used in the present paper), and they belong to *UCPs* family (Crocco et al., 2011; Dato et al., 2012b). Both *UCP2* and *UCP3* confirmed to have a major effect on physical tests such as HG and WS in the whole sample, as well as in the two gender separately, with the rs11235972 influencing the survival at old age too. The association of *UCP3* gene variability with physical exercise is particularly relevant, considering its expression in skeletal muscle, where it regulates fatty acid metabolism, oxidative status, and ROS production, suggesting a correlation between the uncoupling process and the regulation of muscle metabolism/catabolism in the elderly (Dato et al., 2012b and references therein).

All the other associations are new and open the investigation of their variability in future studies. Furthermore, associations holding the multiple comparison correction were found in the female sub-sample, suggesting a sex-specific effect of the pro-anti-oxidant pathway on the analyzed functional parameters.

The gene having a major effect on ADL phenotype is *TXNRD1*, both in the whole sample ($p = 0.010$) and specifically in the female sub-group, where the association is very close to significance also after correction for multiple testing ($p = 0.002$). Although borderline, *TXNRD1* was associated with WS in the whole sample too, confirming the influence that this gene can have on functional activity. Thioredoxin (Trx) is a crucial protein for antioxidant defense, is a redox regulator of the intra- and extra-cellular signaling pathways and transcription factors, and contributes in the protein folding through the catalysis of sulfur-exchange reactions among protein complexes. Mammalian Trx have been associated with neuro-protection against Alzheimer's and Parkinson's disease (Masutani et al., 2004) and their up-regulation has been suggested as a good strategy for prevention and treatment

of these disabling age-related diseases. The genetic variability of *TXNRD1* (rs10047589) has been found associated with late life survival in a previous work from our group (Soerensen et al., 2012b); the present work confirms the association of *TXNRD1* gene with survival in this cohort, in particular in the female population ($p = 0.024$), adding the evidence that the gene can have a role in the quality of aging too, at least in the Danish population.

A major effect on ADL score was also observed for the *UQCRCF1* gene in the male sample, which unfavorable association with ADL in the sub-sample converges in a correlation with a lower survival chance in the whole sample studied. This protein, Ubiquinol-Cytochrome C Reductase, Rieske iron-sulfur polypeptide 1, is a subunit of Complex III of the mitochondrial respiratory chain. Its variability has never been investigated in relation to human longevity and related phenotypes; however, considering that the proper functioning of mitochondrial respiration is crucial for the cellular homeostasis and its de-regulation can increase oxidative stress, the observed result suggests the importance of an efficient oxidative phosphorylation for maintaining a good level of activity and independence in daily occupations after the ninth decade of life.

In the whole sample as in the two genders separately, a broadly protective role for the maintenance of a good functional status seems to be deserved to the glutathione (GSH) sub-pathway. In particular, genes belonging to the GSH sub-pathway were associated with WS (*GCLC*) and MMSE levels (*GLRX* and *GSTP1*) in the whole sample. In males, *GPX4* and *GCLC* variability were found associated with ADL and HG strength, respectively. In females, the association between *GLRX* and HG hold also for the multiple comparison correction ($p = 0.001$), further suggesting that GSH metabolism can have an effect on functional status at old age. Such an effect may be linked to oxidative stress response, but also to other cellular mechanisms controlled by this metabolic way. The genes found associated with functional status in this work belong to the Free -Radical Induced Apoptosis pathway (http://www.biocarta.com/pathfiles/h_freePathway.asp) activated in endothelial cells as a response to inflammation-related oxidative stress and playing a role in susceptibility to cancer, and other diseases. As an example, polymorphisms of *GSTP1* were found associated with different cancers and chemotherapy response (Sekine et al., 2007; Strange and Fryer, 1999), while mutations at *GCLC* locus have been associated with myocardial infarction (Koide et al., 2003). Furthermore, the apoptotic events activated by GSH sub-pathway in endothelial cells for resisting to oxidative stress have been involved in the development and progression of atherosclerosis (Ballatori et al., 2009), another clinical conditions typical of old age. On the whole, this is the first work demonstrating an influence of GSH sub-pathway in age-related non pathological phenotypes, which may influence the quality of aging.

Non negligible effects on physical functioning were also suggested for *NDUFS1*, which variability was found related to HG and WS in the whole sample. The strong association ($p = 0.009$) of the same gene with survival in the female subsample suggests that *NDUFS1* gene, codifying for the largest subunit of mitochondrial complex 1, and which mutations were correlated with inefficiency of oxidative phosphorylation in Leigh Syndrome (Martín et al., 2005), can have a role also in the preservation of physical functioning at old age and lastly influence survival.

Furthermore, very interesting is the association with MMSE levels ($p = 0.005$) observed in the whole sample for *MT1A* variability, overall found to be protective for cognitive functioning at SNP level. Considering that the over expression of MT (metallothionein gene, member of a large family of proteins with anti-apoptotic effect) was the only demonstrated to increase mouse lifespan (Swindell, 2011), protecting against obesity and diet-induced oxidative damage, we may argue that these genes would deserve future attention for their relation with longevity and related phenotypes. Borderline effects on cognitive performances have been observed for *NOS3* variability both in the male ($p = 0.052$ with MMSE) and female sample ($p = 0.023$ with CCS), in line with previous evidences indicating an association the gene with mild cognitive impairment (Solé-Padullés et al., 2004). Considering the recent evidence of association between the genetic variability of *NOS1* and *NOS3* genes with depressive symptoms and disability in a population from Southern Italy, finally influencing quality of aging and longevity (Montesanto et al., 2013), the present results suggest that NOS variability can influence age-associated traits and survival also in the Northern European population.

Possible limitations of the present study can be found in the methodology of the gene-based approach itself, which allows to identifying an effect on specific phenotypes but lacks in establishing the direction of this effect. For such a reason, favorable or unfavorable effects can be deduced at single SNP level only. Furthermore, the male sample is may be undersized respect to the female one: this unbalance in sample composition, although common in association studies with longevity, due to the sex-ratio characterizing long-lived cohorts (Passarino et al., 2002), can explain the inconsistent findings between the two genders, when separately considered.

5. Conclusions

In conclusion, this work supports a role of the pro-anti-oxidant pathway in physical and cognitive status after the ninth decade of life, particularly evidencing the effect of some sub-pathways, as UCP, Glutathione and thioredoxin pathways for the maintenance of functional status at old age. The role of these genes in the preservation of physical and cognitive status may be influenced by gender, suggesting possible interactions with endocrine systems, in line with recent evidences indicating a role of oxidative stress in the aging of the endocrine system, converging on the induction or regulation of inflammatory status typical of old age (Vitale et al., 2013). In addition, a new association was reported in relation to MMSE levels, related to metallothionein gene *MT1A*, which open new ways of investigations of this gene family in relation to cognitive functioning in humans.

It is clear that final conclusions on the role of the genetic variants on stress mechanisms can be drawn only after confirmation by functional analyses. However, we believe that all the associations found in this study deserve attention for future replications studies.

In general, the variability of genes affecting stress response in different individuals and populations deserve future studies. In fact, although oxidative stress pathway has been extensively characterized from a biochemical and molecular point of view, thanks to experimental models and knock out approaches, population association studies investigating

the role of genetic variability in genes coding for proteins belonging to the oxidative stress cascade, as key point regulators such as NFkB or MAP kinases, are still scarce. Instead, a deeper knowledge of the genetic background able to provide a more or less efficient stress response and influence age-associated traits can help to understand the link between stress response and human aging, finally adding a piece to the complex scenario of determinants of human longevity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

General characteristics and post-survey mortality in the Danish 1905-Cohort.

	<u>Men</u>	<u>Women</u>	<u>Total</u>
	(n = 313)	(n = 776)	(n = 1089)
Survival			
Deaths [*] [n (%)]	382 (97.9)	963 (97.3)	1345 (97.5)
Person-Years	1244	3990	5234
Mortality rate per 100	30.7	24.1	25.6
Age (year)			
Mean (SD)	93.2 (0.3)	93.1 (0.3)	93.2 (0.3)
Range	92.7–93.8	92.7–93.8	92.7–93.8
HG strength [kg]			
Mean (SD)	23.2 (6.5)	13.6 (4.4)	16.3 (6.7)
Range	3–46	2–29	2–46
ADL ^{**} [n (%)]			
Not disabled	180 (46.2)	318 (32.1)	498 (36.1)
Disabled	210 (53.8)	672 (67.9)	882 (63.9)
Walking speed [m/s]			
Mean (SD)	0.22 (0.09)	0.18 (0.08)	0.19 (0.09)
Range	0.05–0.50	0.05–0.50	0.05–0.50
BMI			
Mean (SD)	24.1 (3.2)	22.9 (4.0)	23.2 (3.8)
Range	15.2–37.6	14.0–74.9	14.0–74.9
MMSE			
Mean (SD)	22.3 (5.9)	21.7 (5.4)	21.8 (5.6)
Range	2–30	0–30	0–30
CCS			
Mean (SD)	0.47 (3.4)	0.25 (3.4)	0.32 (3.4)
Range	–7.47–11.84	–7.47–11.81	–7.47–11.84

ADL, Activity Daily Living; HG, Hand Grip; BMI, Body Mass Index; MMSE, Mini Mental State Examination; CCS, Composite Cognitive Score.

^{*} Survival data are calculated from a 12 year follow-up period.^{**} Participants were defined as “not disabled” if independent in all items and “disabled” if dependent in at least one item.

Table 2

Genes in the pro-anti-oxidant pathway showing a nominally significant association ($p < 0.05$) with at least one trait under study (ADL, HG, WS, MMSE, CCS) plus survival.

Gene	*Effect	numSNPs	Chr	ADL			HG			WS		
				Test stat	P-value	Best SNP	Test stat	P-value	Best SNP	Test stat	P-value	Best SNP
ACOX1	PRO	10	17	2.503	0.131	rs7226127	1.515	0.516	rs17583163	0.958	0.839	rs12430
GCLC	ANTI	14	6	2.183	0.177	rs7742367	2.121	0.188	rs16883912	3.919	0.017	rs670548
GLRX	ANTI	8	5	1.140	0.714	rs3756704	2.714	0.109	rs1047420	1.914	0.355	rs2007
GSTP1	ANTI	4	11	0.668	0.924	rs1695	1.826	0.357	rs1138272	0.954	0.776	rs7941395
LOX	PRO	1	5	0.053	0.957	rs17352686	1.213	0.477	rs17352686	0.165	0.905	rs17352686
MT1A	ANTI	3	16	1.928	0.363	rs4784701	0.419	0.940	rs4784701	1.051	0.687	rs8049883
NDUFS1	OXPHOS	5	2	0.898	0.790	rs11695633	4.196	0.022	rs6435324	4.431	0.025	rs6435326
NDUFV1	OXPHOS	1	11	0.570	0.701	rs12793832	2.863	0.179	rs12793832	0.092	0.949	rs12793832
PRDX3	ANTI	6	10	2.307	0.228	rs11198811	3.515	0.076	rs3377	0.425	0.955	rs4752257
SOD2	ANTI	5	6	2.503	0.164	rs911847	1.573	0.464	rs5746136	3.230	0.085	rs2842980
TXNRD1	ANTI	15	12	4.157	0.010	rs7310505	1.100	0.784	rs7301631	3.252	0.053	rs7310505
UCP2	ANTI	4	11	1.225	0.588	rs2632725	1.807	0.350	rs7109266	4.706	0.032	rs659366
UCP3	ANTI	4	11	1.154	0.624	rs3781907	4.868	0.018	rs11235972	4.715	0.027	rs1685354
UQCRCF1	OXPHOS	4	19	2.272	0.240	rs10420904	0.619	0.888	rs759628	1.936	0.357	rs759628
Survival												
ACOX1	PRO	10	17	1.276	0.663	rs7219716	3.105	0.048	rs8065144	1.201	0.738	rs3643
GCLC	ANTI	14	6	0.887	0.887	rs534957	1.197	0.699	rs534957	1.620	0.438	rs2397147
GLRX	ANTI	8	5	3.382	0.041	rs871775	1.689	0.411	rs4561	0.820	0.909	rs1047420
GSTP1	ANTI	4	11	5.446	0.009	rs1695	2.778	0.126	rs1138272	2.558	0.171	rs7927381
LOX	PRO	1	5	1.052	0.532	rs17352686	3.034	0.167	rs17352686	7.597	0.013	rs17352686
MT1A	ANTI	3	16	6.231	0.006	rs4784701	1.306	0.546	rs8049883	1.356	0.543	rs4784701
NDUFS1	OXPHOS	5	2	1.260	0.588	rs11695633	0.204	0.998	rs6435326	2.236	0.242	rs6435324
NDUFV1	OXPHOS	1	11	9.613	0.005	rs12793832	4.422	0.079	rs12793832	0.178	0.893	rs12793832
PRDX3	ANTI	6	10	4.068	0.049	rs1553850	3.718	0.065	rs3740562	0.742	0.811	rs3377
SOD2	ANTI	5	6	1.264	0.615	rs5746136	0.812	0.840	rs5746151	4.553	0.012	rs2758331

Gene	*Effect	numSNPS	Chr	ADL			HG			WS		
				Test stat	P-value	Best SNP	Test stat	P-value	Best SNP	Test stat	P-value	Best SNP
TXNRD1	ANTI	15	12	1.779	0.371	rs4964778	1.896	0.308	rs4964735	2.257	0.184	rs7310505
UCP2	ANTI	4	11	1.019	0.685	rs2632725	1.093	0.666	rs659366	1.823	0.359	rs659366
UCP3	ANTI	4	11	2.048	0.285	rs11235972	0.990	0.699	rs647126	2.566	0.182	rs11235972
UQCRES1	OXPPOS	4	19	4.115	0.038	rs759628	2.527	0.178	rs759628	4.094	0.038	rs759628

Association showing a significance below $p < 0.05$ are indicated in bold.

* Note: The prevalent effect of the different genes inside the pro-anti-oxidant metabolic pathway was reported. PRO: pro-oxidant; ANTI: anti-oxidant; OXPPOS: involved in mitochondrial oxidative phosphorylation.

Table 3

Genes showing significant association at a nominal level of 0.05 with at least one trait under study (ADL, HG, WS, MMSE, CCS) plus survival, in nonagenarian males (a) and females (b) from the Danish 1905 Cohort.

a) Males			
Gene	Best SNP	F statistic	P-value
<i>ADL</i>			
GPX4	rs4588110	4.174	0.045
SOD3	rs17878863	4.114	0.031
UQCRFS1	rs10420904	6.845	0.002
XDH	rs1429372	3.185	0.020
<i>HG</i>			
GCLC	rs2397147	3.413	0.024
<i>WS</i>			
UCP2	rs659366	6.125	0.009
<i>MMSE</i>			
NOS3	rs743506	101.535	0.050
<i>CCS</i>			
CP	rs9853335	3.222	0.021
CYC1	rs11780874	5.141	0.022
b) Females			
Gene	Best SNP	F statistic	P-value
<i>ADL</i>			
TXNRD1	rs7310505	4.979	0.002
TXN2	rs12159295	3.990	0.016
ACOX1	rs3643	3.186	0.039
<i>HG</i>			
GLRX	rs6556884	5.750	0.001
UCP3	rs11235972	6.619	0.002
UCP2	rs7109266	5.650	0.008
NOX1	rs5921669	3.752	0.024
<i>WS</i>			
SOD2	rs2842980	5.103	0.005
GCLC	rs2100375	4.159	0.007
<i>MMSE</i>			
MT1A	rs4784701	7.717	0.002
<i>CCS</i>			
ACOX1	rs8065144	3.176	0.046
NOS3	rs2853792	3.471	0.023
<i>Survival</i>			
NDUFS1	rs6435324	5.115	0.009
SOD2	rs911847	3.715	0.035

a) Males			
Gene	Best SNP	F statistic	P-value
TXNRD1	rs10861169	3.587	0.024
UCP3	rs11235972	4.004	0.041