Mechanisms of Ageing and Development

Article *in* Mechanisms of ageing and development · August 2015

DOI: 10.1016/j.mad.2015.08.003 · Source: PubMed

7 authors, including:

Giuseppina Rose
Università della Calabria
127 PUBLICATIONS **3,309** CITATIONS
SEE PROFILE

Paolina Crocco
Università della Calabria
53 PUBLICATIONS **622** CITATIONS
SEE PROFILE

Francesco De Rango
Università della Calabria
55 PUBLICATIONS **1,069** CITATIONS
SEE PROFILE

Andrea Corsonello
INRCA Istituto Nazionale di Ricovero e Cura per Anziani
275 PUBLICATIONS **5,729** CITATIONS
SEE PROFILE

Mechanisms of Ageing and Development 150 (2015) 27–33

Contents lists available at ScienceDirect

Mechanisms of Ageing and Development

journal homepage: www.elsevier.com/locate/mechagedev

Original article

Metabolism and successful aging: Polymorphic variation of syndecan-4 (*SDC4*) gene associate with longevity and lipid profile in healthy elderly Italian subjects

Giuseppina Rose[a,*,1], Paolina Crocco[a,1], Francesco De Rango[a], Andrea Corsonello[c], Fabrizia Lattanzio[d], Maria De Luca[b], Giuseppe Passarino[a]

[a] *Department of Biology, Ecology and Earth Science, University of Calabria, Rende 87036, Italy*
[b] *Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, AL 35294, USA*
[c] *Italian National Research Centre on Ageing, Cosenza I-87100, Italy*
[d] *Scientific Direction of the Italian National Research Centre on Ageing, Ancona 6100, Italy*

ARTICLE INFO

Article history:
Received 20 June 2015
Received in revised form 27 July 2015
Accepted 3 August 2015
Available online 5 August 2015

Keywords:
Aging
Longevity
Extracellular matrix
Metabolism
Syndecans

ABSTRACT

Evidences from model systems and humans have suggested that genetic alterations in cell–ECM interactions and matrix-mediated cellular signaling cascades impact different aspects of metabolism and thereby life span. In this frame, a genetic variant (rs1981429) in the *SDC4* gene encoding for syndecan-4, a central mediator of cell adhesion, has been associated with body composition in children and coronary artery disease in middle-age subjects. In order to test the hypothesis that syndecans might affect life span by affecting metabolic endophenotypes, 11 SNPs within the *SDC4* gene were tested for association with longevity in a cohort of 64–107 aged individuals. We then determined whether the longevity-associated SNPs were correlated with metabolic parameters in the age group 64–85 years. RobustSNP association tests showed that rs1981429 was negatively associated with longevity (Theo$p$ = 0.028), but also with high levels of triglyceride (Theo$p$ = 0.028) and low levels of low-density lipoprotein-cholesterol (LDL-C) (Theo$p$ = 0.009). On the other hand, rs2251252 was found to be positively correlated with longevity (Theo$p$ = 0.018) and high LDL-C (Theo$p$ = 0.022). On the whole, our results suggest that *SDC4* alleles affect lipid profile in elderly subjects and may in part mediate the link between LDL-C and longevity.

1. Introduction

Genetic differences among individuals explain as much as 20–30% of the variability in life span in natural populations (Finch and Tanzi, 1997; Hjelmborg et al., 2006). Genes that were shown to be critical in modulating life span include those involved in cellular maintenance and repair, oxidative stress response, and metabolic pathways, especially those related to nutrient-sensing signaling pathways (Barzilai et al., 2012; Montesanto et al., 2012; Murabito et al., 2012; Dato et al., 2013; Moskalev et al., 2014). Within the metabolism related genes, the application of data mining/systems biology approaches to aging research has provided intriguing evidence suggesting that proteins involved in cell–cell and cell-extracellular matrix (ECM) interactions may be associated with longevity and age-related disease (Wolfson et al., 2009).

The ECM is an intricate network of macromolecules that provides structural and anchoring support to cells and controls many aspects of the cell's dynamic behavior through adhesion proteins, such as integrins and syndecans (SDCs) (Daley et al., 2008). SDCs belong to the family of heparan sulfate proteoglycans (HSPGs) that also include glycosylphosphatidylinositol-anchored glypicans and secreted proteoglycans found in the ECM (e.g., agrin, collagen XVIII, and perlecan) (Morgan et al., 2007; Xian et al., 2010). Across species, all HSPGs are characterized by an extracellular domain with attachment sites for heparan sulfate polysaccharide chains that mediate interactions with a wide array of ligands, including soluble growth factors, morphogens, chemokines, and cytokines (Morgan et al., 2007; Xian et al., 2010). However, unlike the other HSPGs, SDCs also contain highly conserved transmembrane domains and short cytoplasmic tails. These cytoplasmic domains allow SDCs to function independently and in synergy with integrin-mediated signaling to control cell adhesion and behavior (Morgan et al., 2007; Xian et al., 2010).

Given that signaling between cells and the ECM plays a pivotal role in tissue/organ homeostasis (Bonnans et al., 2014), it is perhaps

* Corresponding author. Fax: +39 0984493601.
E-mail address: pina.rose@unical.it (G. Rose).
[1] Giuseppina Rose and Paolina Crocco equally contributed to this study.

http://dx.doi.org/10.1016/j.mad.2015.08.003
0047-6374/

not surprising that genetic perturbation of these interactions might influence life span. To this end, De Luca et al. (2010) and Wilson et al. (2013) recently reported that hypomorphic mutations in the Drosophila *Sdc* gene reduce life span in flies. Notably, *Sdc* mutant flies also display less fat and glycogen reserves and lower metabolic activity than control flies (De Luca et al., 2010), suggesting that Sdc might influence life span through a role in metabolism.

Unlike invertebrates, humans contain four *SDC* genes. Three of them, *SDC1*, *SDC2*, and *SDC3*, are expressed in a tissue-specific manner, whereas the fourth, *SDC4*, is expressed in a variety of cell types (Xian et al., 2010). Previously, common genetic variants in the human *SDC4* gene were significantly associated with adiposity, fasting glucose, and energy expenditure in American children (De Luca et al., 2010). Association analysis in a cohort of middle-aged Finnish subjects also reported associations between *SDC4* rs1981429 and hypertension, body mass index (BMI), and coronary heart disease (CHD) (Kunnas and Nikkari, 2014). It is well established that the ectodomain of all SDCs can be released from the cell surface by metalloproteinase-mediated cleavage (Fitzgerald et al., 2000). Once released, the ectodomain can compete for ligand binding with the remaining cell-surface SDCs (Fitzgerald et al., 2000). Recent *in vitro* studies have implicated insulin in the control of SDC4 ectodomain shedding from adipocytes (Reizes et al., 2006). These studies also indicate that shed adipocyte syndecans associate with the lipoprotein lipase and stabilize its activity, highlighting the importance of syndecans in lipid metabolism (Reizes et al., 2006). Based on these observations, the findings in *D. melanogaster,* and the human genetic studies mentioned above, we reasoned that genetic variation in *SDC4* might affect inter-individual differences in metabolic risk factors and thereby influence the odds of becoming a centenarian. Thus, we investigated this idea in the frame of a collaborative study aimed at analyzing the effect of ECM on metabolism and on metabolic impairments. In particular we tested a total of 11 SNPs spanning the *SDC4* gene for association with longevity in a cohort of healthy elderly subjects (aged 64–107). We then determined whether the SNPs found to be associated with old age were also associated with risk factors of CHD, including BMI, lipid profile, and HbA1c, in a subgroup of elderly subjects (64–85 years of age).

2. Methods

2.1. Study subjects

The study was carried out in a cohort of 624 unrelated subjects (287 males and 337 females, age range 64–107 years; median ages 83.56 (±12.15) and 87.48 (±11.12) years, respectively). All subjects were born in Calabria (South Italy) and their parents and grandparents were native of the same area; samples were collected within the framework of several recruitment campaigns carried out for monitoring the quality of aging in the whole Calabria region from 1999 onwards. Details of the recruitment process were reported in Crocco et al. (2015). All subjects included in the present study did not manifest any major age-related pathology (e.g., cancer, type-2 diabetes, and cardiovascular diseases). White blood cells (WBC) from blood buffy coats were used as source of DNA. The recruitment campaigns received the approval of the Ethical committee of the University of Calabria, Italy. A written informed consent was obtained from all participants before enrolling in the study.

2.2. Phenotypic measurements

Phenotypic measurements included height, weight, BMI and serum lipid risk factors for CHD: total cholesterol (TC), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), and triglycerides (TG). HbA1c was chosen as a clinical marker of CHD because levels of HbA1c between 6.0 and 6.5% were previously associated with increased risk of CHD among healthy, non-diabetic subjects (Pai et al., 2013). Height and weight were measured while subjects were dressed in light indoor clothes and without shoes. Blood samples were withdrawn after 12-h overnight fast and biochemical measurements were performed at the Italian National Research Centre on Ageing (Cosenza) using standard protocols as described elsewhere (Garasto et al., 2003).

2.3. SNPs selection and genotyping

SNPs within the *SDC4* gene were prioritized by a tagging approach, attempting to pick those most likely to be of functional relevance (for instance nonsynonymous SNPs or SNPs located in the 5′ and 3′ UTR regions). SNPs previously investigated in association studies were also selected. Public databases (http://www.ncbi.nih.gov/; http://www.hapmap.org/) were used to collect information about SNPs. SNPs with a minor allele frequency (MAF) less than 10% were excluded from the analysis. The selected SNPs are reported in Table S1.

The SNP's genotypes were determined by Sequenom MassARRAY method (Sequenom iPLEX Assay; Sequenom, San Diego, CA, USA) according to the manufacturer's instruction. Sequenom MassARRAY Assay Designer software (version 3) was used to design primers for PCR and single base extension. Multiplex reactions were used in the whole process. Standard procedures were used to amplify PCR products, PCR production purification with the shrimp alkaline phosphatase (SAP) and a primer extension reaction using the mass extension primer and the terminator. The primer extension products were then desalted on resin, and spotted onto the 384-element SpectroCHIP (Sequenom) for MALDI-TOF analysis using SpectroACQUIRE v3.3.1.3 (Sequenom). Spectra were analyzed using MassARRAY Typer v 4.0.Software (Sequenom). Approximately 8% of the samples were analyzed in duplicate, and the concordance rate of the genotypes was higher than 99%. Eleven of the 12 SNPs analyzed showed call rate higher than 95%; however, genotyping completion rate was lower than 65% for rs6104125.

2.4. Statistical analyses

The analyses were carried out by dividing the sample in cases (350 subjects, age >85 years; median 92.25 ± 4.02) and controls (274 subjects, age $\leqq$85 years; 73.60 ± 5.70). For each *SDC4* SNP, allele frequencies were estimated by counting the observed genotypes. Hardy–Weinberg equilibrium was assessed using the exact test proposed by Wigginton et al. (2005). Standard errors for alleles were computed according to the hypothesis of the multinomial distribution. The RobustSNP algorithm, implemented in the R package, which is a robust association test suitable for quantitative and binary traits (So and Sham, 2011), was used to test the association between each *SDC4* SNP and the trait of interest using different genetic models (additive, dominant, and recessive). We denote the minor allele as the effect allele and the major allele as the non-effect allele. In regression models, the genotype was coded as the number of the minor allele (0, 1, 2) in the additive model, whereas in the dominant and recessive models the genotype was coded as 0 or 1 depending on whether the subject carried the minor allele (genotype = 1 if minor allele carrier, 0 if not [dominant model]; genotype = 1 if homozygous for minor allele, 0 if not, [recessive model]). For each model, a z-score was returned, representing the z-statistics for the regression analyses, with a p model referred to the p value for the most likely genetic model.

The p-value based on the maximum of the three genetic models, adjusted for multiple testing (theo$P$) was obtained. In the regression models used for testing the association between the variability of

analyzed polymorphisms and the human longevity, the variable sex was used as covariate. Age, sex and BMI were used as covariates when analyzing the association with serum lipid concentrations.

D' linkage disequilibrium coefficients were assessed using Haploview 4.2 (Barrett et al., 2005). In order to model the effect of the *SDC4* haplotypes on the probability to attain longevity we used the *haplo.stats* package of R.

2.5. Bioinformatic analyses

To investigate the functions of our candidate SNPs or SNPs in LD ($r^2 \geq 0.8$), we explored the ENCODE (https://genome.ucsc.edu/ENCODE/) (ENCODE Project Consortium et al., 2012) and 1000 Genomes Project (http://www.1000genomes.org/) (McVean and the 1000 Genomes Project Consortium, 2012) data implemented in the public databases HaploReg v3 (http://www.broadinstitute.org/mammals/haploreg/haploreg_v3.php) (Ward and Kellis, 2012) and RegulomeDB (http://www.regulomedb.org/) (Boyle et al., 2012). Moreover, to determine whether SNPs influenced gene expression, we explored the Genotype-Tissue Expression (GTEx) eQTL (Expression Quantitative Trait Loci) database (http://www.ncbi.nlm.nih.gov/gtex/GTEX2/gtex.cgi).

3. Results

3.1. SCD4 association with longevity

The anthropometric and metabolic characteristics of the study subjects are reported in Table 1.

All SNPs were in Hardy-Weinberg equilibrium ($p > 0.05$). First, single SNP analyses were conducted to find associations with longevity. After fitting dominant, recessive, and additive models and adjusting for sex, we found that rs1981429, rs2267871, rs2251252, and rs6130811 were associated with the analyzed phenotype (Table 2). The association between rs2267871 and longevity, however, was not significant after adjusting for multiple testing (Table 2); hence, caution is needed in interpreting this finding. The z-statistics of these models indicated that subjects homozygous for the G allele at rs1981429 (recessive model) have a lower probability to be part of the oldest group (negative score; $p^{\text{Model}} = 0.013$), while carriers of the A allele at rs2251252 and the G allele at rs6130811 (dominant models) showed a higher probability to be part of the oldest group (positive scores; $p^{\text{Model}} = 0.008$ and $p^{\text{Model}} = 0.009$, respectively).

Fig. 1 shows the linkage disequilibrium (LD) pattern among the 11 genotyped SNPs. No significant LD ($r^2 < 0.8$) was detected between markers, except for the pair rs1981429 and rs2228384. The haplotype analysis for these two polymorphic sites revealed that, although the global *p*-value was 0.082, the major haplotype, G (rs1981429)-T (rs2228384), was significantly associated with a decreased probability of attaining longevity (negative score; $p = 0.015$) (Table 3). Thus, haplotype analysis confirmed association results with the single markers.

Next, in the 64-85 year old group of healthy subjects we tested for association between the three SNPs found to be associated with old age and metabolic parameters which are risk factors for several age-related diseases that impart significant morbidity and mortality among elderly individuals. After adjusting for sex, age, and BMI, we found that the minor rs1981429 G allele, negatively correlated with survival after 85 years of age, was also correlated with higher serum triglyceride (TG) levels (positive score; $p^{\text{Additive Model}} = 0.024$). Interestingly, the same G allele was also associated with low LDL-Cholesterol (LDL-C) levels (negative score; $p^{\text{Dominant Model}} = 0.009$) (Table 4). On the other hand, the minor allele A of rs2251252, which was positively associated with longevity, was correlated with higher levels of LDL-C (positive score; $p^{\text{Recessive Model}} = 0.022$). A similar result was found for the minor allele G of rs6130811, but the association was not significant after adjusting for multiple testing (Table 4). Finally, there was no significant association between any of the three longevity-SDC4 polymorphisms with BMI or HbA1c (Table 4).

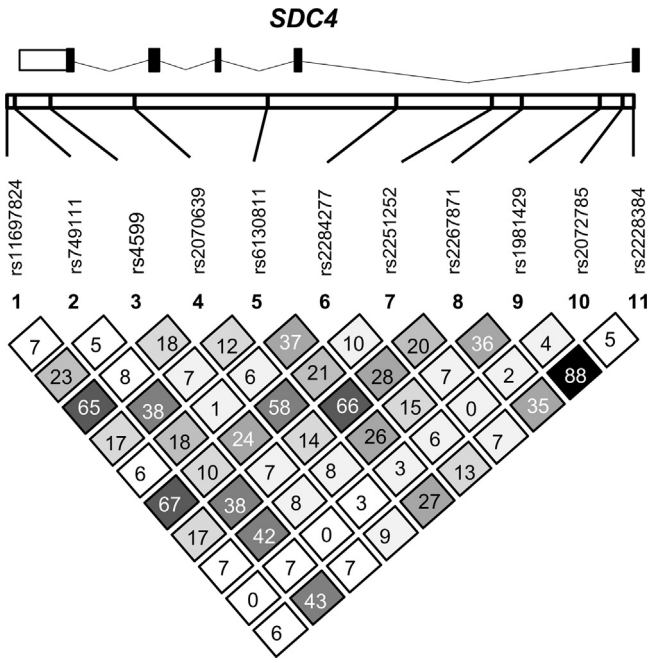

Fig. 1. Schematic diagram showing the location and linkage disequilibrium pattern of the *SDC4* SNPs genotyped in a cohort of Italian elderly subjects. Numerical values shown correspond to r^2 values.

3.2. Exploring the functional consequences of SDC4 variants

Given that the phenotype-associated SNPs are all intronic, it is possible that they are not causal variants but are in LD with the functional polymorphism(s) elsewhere in the genome. However, we cannot exclude the possibility that these SNPs regulate gene activity at the transcript level. Therefore, we used regulatory information from ENCODE data using HaploReg v3 and RegulomeDB databases (Ward and Kellis, 2012; Boyle et al., 2012) to investigate the possible functional impact of these SNPs and their proxies in high LD ($r^2 \geq 0.8$ in Europeans from the 1000 Genomes Project). Detailed results for all analyzed SNPs are given in Table S2. LD patterns showed that the SNPs in LD with the phenotype-associated SNPs rs1981429, rs2251252, and rs6130811 were all in non-coding regions of *SDC4*. Additionally, we found unlike the other SNPs in LD with rs1981429, rs1981429 itself has functional implication since it was previously reported to be an eQTL-SNP in the liver of Caucasians (Schadt et al., 2008). Furthermore, it acts as a trans-eQTL associated with changes in the expression of the *retinoid X receptor gamma* (*RXRG*) gene, which is located on chromosome 1 (Schadt et al., 2008). The functional implication of rs1981429 is further corroborated by RegulomeDB results showing that it is "likely to affect binding and linked to expression of a gene target" (score 1f). As for rs1981429, SNP rs2251252, which is in LD with only one other SNP, is also likely to be of functional importance as it was classified as "likely to affect binding" (score 2b).

Twenty-three SNPs were identified that were proxies with the phenotype-associated SNP rs6130811 (score 5: minimal binding evidence). Three of them, rs6094129, rs6104120 and rs2267868,

Table 1
Anthropometric and metabolic characteristics of the study cohort stratified according to age groups.

	64–85 year old subjects ($N = 274$)	86–107 year old subjects ($N = 350$)	p-value
Age	73.60 ± 5.70[a]	92.25 ± 4.02	<0.001
n Male/Female	148/126	139/211	<0.001
Height (cm)	161.50 ± 8.99	151.81 ± 9.11	<0.001
Weight (kg)	71.49 ± 13.42	54.61 ± 12.04	<0.001
BMI[b] (kg/m2)	27.33 ± 4.23	23.57 ± 4.11	<0.001
T-Chol[c] (mg/dl)	203.29 ± 42.62	197.41 ± 42.65	0.089
TG[d] (mg/dl)	125.95 ± 55.61	121.45 ± 55.99	0.165
HDL-C[e] (mg/dl)	56.43 ± 14.76	57.72 ± 15.13	0.252
LDL-C[f] (mg/dl)	122.19 ± 35.08	115.94 ± 36.77	0.039
HbA1c[g] (%)	5.67 ± 0.91	5.12 ± 2.72	<0.001

[a] Mean ± SE.
[b] BMI: body mass index.
[c] T-Chol: total cholesterol.
[d] TG: triglyceride.
[e] HDL-C: HDL-cholesterol.
[f] LDL-C: LDL-cholesterol.
[g] HbA1c: Hemoglobin A1c.

Table 2
Results of robust association tests between *SDC4* SNPs and longevity.

SNP	MAF	Z[a].add	Z.rec	Z.dom	P[b].add	P.rec	P.dom	Theop[c]
rs2228384	0.38 (C)	1.821	1.276	1.634	0.069	0.202	0.102	0.140
rs2072785	0.40 (T)	1.270	−0.041	1.881	0.204	0.967	0.060	0.124
rs1981429	0.44 (G)	−1.385	−2.495	0.018	0.166	0.013	0.986	**0.028**
rs2267871	0.30 (T)	−1.761	−0.589	−2.055	0.078	0.556	0.040	0.082
rs2251252	0.47 (A)	2.245	0.950	2.663	0.025	0.342	0.008	**0.018**
rs2284277	0.21 (A)	−0.530	−0.412	−0.457	0.596	0.680	0.648	0.836
rs6130811	0.31 (G)	2.456	0.994	2.609	0.014	0.320	0.009	**0.020**
rs2070639	0.49 (T)	−1.630	−1.443	−1.209	0.103	0.149	0.227	0.203
rs4599	0.21 (G)	−0.690	0.328	−0.985	0.490	0.743	0.325	0.545
rs749111	0.16 (T)	−0.854	−0.744	−0.712	0.393	0.457	0.477	0.630
rs11697824	0.16 (A)	−0.580	−0.073	−0.644	0.562	0.942	0.519	0.769

In bold, p-value significant after adjusting for multiple comparisons.
MAF: Minor Allele Frequency in our control group.
[a] Z-score represents the z-statistics under the additive, recessive and dominant models.
[b] p-value calculated for each genetic model.
[c] Theo$p$ refers to the p-value adjusted for multiple comparisons (due to the three different tested genetic models).

Table 3
Estimation of haplotype frequencies in the *SDC4* SNPs and association with longevity in the analyzed sample.

SNPs	rs2228384	rs1981429	Freq[a]	Score	P-value[b]
	T	G	0.41452	−2.42697	0.01523
	T	T	0.18413	0.97116	0.33147
	C	G	0.01198	1.00192	0.31638
	C	T	0.38937	1.44286	0.14906

Note: p-value of global score statistics based on 10^4 replications was $p = 0.082$.
[a] Estimated haplotype frequency.
[b] Monte-Carlo p-value from 10^4 replications.

had a score of 3a (less likely to affect binding); therefore, we cannot exclude that one of these markers may be the actual causal variant.

4. Discussion

Growing evidence from model systems and humans suggest that genetic alterations in cell–ECM interactions and matrix-mediated cellular signaling cascades impacts the decline of age-dependent physiological performance and thereby life span (Goddeeris et al., 2003; Hansen et al., 2005; Wolfson et al., 2009; De Luca et al., 2010, 2011; Wilson et al., 2013; Kumsta et al., 2014; Nishimura et al., 2014). Consistently, here we report that common genetic variants in the *SDC4* gene affect human longevity. Specifically, we found that Italian elderly subjects homozygous for the G-allele of *SDC4* rs1981429 had a lower likelihood of becoming long-lived than those carrying the T-allele. Furthermore, G allele carriers had higher levels of TG. This is particularly intriguing since we previously showed that American children (age 7–12 years) homozygous for the minor G-allele of rs1981429 had more intra-abdominal fat and less lean tissue mass (De Luca et al., 2010). Data on visceral fat were not available in the Italian cohort so we could not test for association between this trait and rs1981429. However, the association of rs1981429 G-allele with high serum TG levels, which is one of the major risk factors for cardiovascular disease (Miller et al., 2011), suggests that this *SDC4* genetic variant might predispose to disease and reduce longevity. Somewhat counter intuitive is the fact that the same G allele at rs1981429 had opposite direction effect for LDL-C, given that high levels of LDL-C rather than low levels are generally associated with increased mortality in middle-aged individuals. However, epidemiological studies of older populations have led to conflicting conclusions on the relationship between LDL-C and all-cause mortality. For instance, some studies reported that high levels of LDL-C were associated with increased risk for CHD morbidity and mortality or all-cause mortality, while other studies found association between low levels of LDL-C and increased risk of death in elderly people (Aronow and Ahn, 1994; Schupf et al.,

Table 4
Results of the RobustSNP association test with the hematological variables (total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol, Fasting glucose and Hemoglobin A1c) in the control group.

	SNP	Z[a].add	Z.rec	Z.dom	P[b].add	P.rec	P.dom	Theop[c]
Total cholesterol	rs1981429	−1.496	−0.315	−2.091	0.135	0.753	0.036	0.077
	rs2251252	1.043	1.948	−0.029	0.297	0.051	0.977	0.108
	rs6130811	1.148	1.612	0.566	0.251	0.107	0.572	0.212
Triglyceride	**rs1981429**	**2.251**	**1.779**	**2.168**	**0.024**	**0.075**	**0.030**	**0.049**
	rs2251252	−1.238	−0.716	−1.291	0.216	0.474	0.197	0.356
	rs6130811	−1.167	−1.862	−0.659	0.243	0.063	0.510	0.129
HDL cholesterol	rs1981429	−0.451	0.461	−1.087	0.652	0.645	0.277	0.463
	rs2251252	0.479	0.429	0.372	0.632	0.668	0.710	0.859
	rs6130811	0.665	0.709	0.463	0.506	0.478	0.643	0.715
LDL cholesterol	**rs1981429**	**−2.318**	**−1.226**	**−2.626**	**0.020**	**0.220**	**0.009**	**0.020**
	rs2251252	**1.397**	**2.288**	**0.241**	**0.162**	**0.022**	**0.809**	**0.049**
	rs6130811	1.369	1.981	0.675	0.171	0.048	0.499	0.101
Fasting glucose	rs1981429	−0.306	−0.098	−0.382	0.760	0.922	0.703	0.907
	rs2251252	0.064	0.994	−0.909	0.949	0.320	0.363	0.528
	rs6130811	0.294	0.877	−0.400	0.769	0.381	0.689	0.603
Hemoglobin A1c	rs1981429	−0.198	−0.190	−0.140	0.843	0.849	0.889	0.975
	rs2251252	−0.728	0.524	−1.446	0.467	0.600	0.148	0.274
	rs6130811	−0.008	0.855	−0.748	0.993	0.392	0.455	0.614

In bold, *p*-value significant after adjusting for multiple comparisons.
[a] Z-score represents the *z*-statistics under the additive, recessive and dominant models.
[b] *p*-value calculated for each genetic model.
[c] Theo*p* refers to the *p*-value adjusted for multiple comparisons (due to the three different tested genetic models).

2005; Tikhonoff et al.., 2005; Razzolini et al., 2007; Cabrera et al., 2012; Bathum et al., 2013; Charach et al., 2014). Consistently, high levels of cholesterol and LDL-C were reported to predict survival in the oldest old (Cevenini et al., 2014). Based on these observations, our results imply that high levels of LDL-C in elderly subjects benefit their longevity and that *SDC4* alleles might mediate this relationship. This is further corroborated by the fact that the *SDC4* rs2251252-A allele, which we report increasing the likelihood of become a centenarian, was also associated with higher levels of LDL-C.

It is important mentioning that we are aware that our results, as most of those regarding association studies, will need further support from additional studies, possibly with larger population samples. However, it is of note that our study was "hypothesis driven", that all the results obtained were in keeping with previous results and, consequently, with the driving hypothesis of the study and, finally, that the population sample has been collected from a selected population, quite homogeneous where there has been no significant immigration in the last century and therefore the chances of age related stratifications are limited. An additional limitation of the study is the lack of an *in vitro* evidence of functionality of the *SDC4* SNPs correlated with longevity and metabolic phenotypes. However, the *in silico* functional prediction for these SNPs and their LD proxies provided significant findings. One interesting result was that all the SNPs in LD with our associated variants were located in the *SDC4* gene, suggesting a direct role of *SDC4* in the traits analyzed. Moreover, the *in silico* analysis revealed that *SDC4* rs1981429 could have a potential regulatory role (Table S2). Specifically, previous work by Schadt et al. (2008) reported that rs1981429 might function as a trans-eQTL SNP associated with the expression of the *Retinoic acid receptor-gamma* (*RXRG*) gene expression in the liver of Caucasians. *RXRG* encodes a member of the RXR nuclear receptors that function as metabolic regulators to control a variety of physiological processes (Lefebvre et al., 2010). For example, RXR heterodimerizes with the 1,25-dihydroxyvitamin D-activated vitamin D receptor (VDR) to stimulate the expression of genes responsible for vitamin D-induced phenotypes (Pike and Meyer, 2010). Interestingly, interactions between *RXRG* alleles and alleles at the *VDR* locus have been recently shown to influence variability in lipid outcomes and waist-hip ratio in a British cohort (Vimaleswaran et al., 2014). In addition, genetic variants in the *RXRG* gene were significantly associated with familial combined hyperlipidemia (Sentinelli et al., 2013). Robust epidemiological data also support a role of vitamin D in reducing the incidence of cardiovascular morbidity and mortality (Gunta et al., 2013). Thus, the potential relationship between *SDC4* and Vitamin D via *RXRG*, suggested by our findings, could also help explain why the rs1981429 T-allele (instead of the G-allele) was found associated with CHD in the Finnish cohort (Kunnas and Nikkari, 2014). Because of inadequate sunlight exposure in wintertime, vitamin D insufficiency is common in northern countries (Laaksi et al., 2006) and thereby different mechanisms by which the *SDC4* alleles might have become linked to metabolic risk factors are plausible. We found very interesting that also the DNA region containing the rs2251252 was potentially bound by Retinoic acid receptor-alpha (RXRA), and had the potential to alter the affinity of binding for the VDR. Hence, it is quite likely that *RXR-VDR* represents the molecular link between *SDC4* and lipid profile.

In conclusion, taken together, our results unveil a role for *SDC4* in lifespan regulation that is likely dependent on its function on the metabolic control of lipid homeostasis. The exact mechanism behind our genetic findings is unknown to date. However, considering the consistent finding across widely phylogenetically distant species of a role for SDCs in life span determination, our results strongly motivate further studies to determine how *SDC4* genotypes can affect human health span and longevity.

Conflict of interest

All authors approved the final manuscript and none of the authors had any personal or financial conflict of interest.

Acknowledgements

This work was supported by the European Union's FP7 (grant number 259679) and by PON (01_00937)—MIUR "Modelli sperimentali biotecnologici integrati per lo sviluppo e la selezione di molecole di interesse per la salute dell'uomo".

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.mad.2015.08.003.

References

Aronow, W.S., Ahn, C., 1994. Correlation of serum lipids with the presence or absence of coronary artery disease in 1793 men and women aged ≥62 years. Am. J. Cardiol. 73, 702–703, http://dx.doi.org/10.1016/0002-9149(94)90938-5.

Barrett, J.C., Fry, B., Maller, J., Daly, M.J., 2005. Haploview analysis and visualization of LD and haplotype maps. Bioinformatics 21, 263–265, http://dx.doi.org/10.1093/bioinformatics/bth457.

Barzilai, N., Huffman, D.M., Muzumdar, R.H., Bartke, A., 2012. The critical role of metabolic pathways in aging. Diabetes 61, 1315–1322, http://dx.doi.org/10.2337/db11-1300.

Bathum, L., Depont, C., hristensen, R., Engers, P., edersen, L., Lyngsie, P., edersen, P., Larsen, J., Nexøe, J., 2013. Association of lipoprotein levels with mortality in subjects aged 50 + without previous diabetes or cardiovascular disease: a population-based register study. Scand. J. Prim. Health Care 31 (3), 172–180, http://dx.doi.org/10.3109/02813432.2013.824157.

Bonnans, C., Chou, J., Werb, Z., 2014. Remodelling the extracellular matrix in development and disease. Nat. Rev. Mol. Cell Biol. 15, 786–801, http://dx.doi.org/10.1038/nrm3904.

Boyle, A.P., Hong, E.L., Hariharan, M., Cheng, Y., Schaub, M.A., Kasowski, M., Karczewski, K.J., Park, J., Hitz, B.C., Weng, S., Cherry, J.M., Snyder, M., 2012. Annotation of functional variation in personal genomes using RegulomeDB. Genome Res. 22, 1790–1797, http://dx.doi.org/10.1101/gr.137323.112.

Cabrera, M.A., de Andrade, S.M., Dip, R.M., 2012. Lipids and all-cause mortality among older adults: a 12-year follow-up study. Sci. World J. 2012, 930139, http://dx.doi.org/10.1100/2012/930139.

Cevenini, E., Cotichini, R., Stazi, M.A., Toccaceli, V., Palmas, M.G., Capri, M., De Rango, F., Dato, S., Passarino, G., Jeune, B., Franceschi, C., 2014. GEHA project consortium. Health status and 6 years survival of 552 90+ Italian sib-ships recruited within the EU Project GEHA (GEnetics of Healthy Ageing). Age (Dordr.) 36 (2), 949–966, http://dx.doi.org/10.1007/s11357-013-9604-1.

Charach, G., Rabinovich, A., Ori, A., Weksler, D., Sheps, D., Charach, L., Weintraub, M., George, J., 2014. Low levels of low-density lipoprotein cholesterol: a negative predictor of survival in elderly patients with advanced heart failure. Cardiology 127 (1), 45–50, http://dx.doi.org/10.1159/000355164.

Crocco, P., Montesanto, A., Passarino, G., Rose, G., 2015. Polymorphisms falling within putative miRNA target sites in the 3′UTR region of SIRT2 and DRD2 genes are correlated with human longevity. J. Gerontol. Ser. A: Biol. Sci. Med. Sci., http://dx.doi.org/10.1093/gerona/glv058.

Daley, W.P., Peters, S.B., Larsen, M., 2008. Extracellular matrix dynamics in development and regenerative medicine. J. Cell Sci. 121, 255–264, http://dx.doi.org/10.1242/jcs.00604.

Dato, S., Crocco, P., D'Aquila, P., de Rango, F., Bellizzi, D., Rose, G., Passarino, G., 2013. Exploring the role of genetic variability and lifestyle in oxidative stress response for healthy aging and longevity. Int. J. Mol. Sci. 14 (8), 16443–16472, http://dx.doi.org/10.3390/ijms140816443.

De Luca, M., Klimentidis, Y.C., Casazza, K., Chambers, M.M., Cho, R., Harbison, S.T., et al., 2010. A conserved role for syndecan family members in the regulation of whole-body energy metabolism. PLoS One 5, e11286, http://dx.doi.org/10.1371/journal.pone.0011286.

De Luca, M., Crocco, P., Wiener, H., Tiwari, H.K., Passarino, G., Rose, G., 2011. Association of a common LAMA5 variant with anthropometric and metabolic traits in an Italian cohort of healthy elderly subjects. Exp. Gerontol. 46 (1), 60–64, http://dx.doi.org/10.1016/j.exger.2010.10.003.

PENCODE Project Consortium, et al., 2012. An integrated encyclopedia of DNA elements in the human genome. Nature 489, 57–74, http://dx.doi.org/10.1038/nature11247.

Finch, C.E., Tanzi, R.E., 1997. Genetics of aging. Science 278, 407–411.

Fitzgerald, M.L., Wang, Z., Park, P.W., Murphy, G., Bernfield, M., 2000. Shedding of syndecan-1 and -4 ectodomains is regulated by multiple signaling pathways and mediated by a TIMP-3-sensitive metalloproteinase. J. Cell Biol. 148 (4), 811–824, http://dx.doi.org/10.1083/jcb.148.4.811.

Garasto, S., Rose, G., Derango, F., Berardelli, M., Corsonello, A., Feraco, E., Mari, V., Maletta, R., Bruni, A., Franceschi, C., Carotenuto, L., De, B., enedictis, G., 2003. The study of APOA1, APOC3 and APOA4 variability in healthy ageing people reveals another paradox in the oldest old subjects. Ann. Hum. Genet. 67, 54–62, http://dx.doi.org/10.1046/j.1469-1809.2003.00008.x.

Goddeeris, M.M., Cook-Wiens, E., Horton, W.J., Wolf, H., Stoltzfus, J.R., Borrusch, M., Grotewiel, M.S., 2003. Delayed behavioural aging and altered mortality in Drosophila beta integrin mutants. Aging Cell 2, 257–264, http://dx.doi.org/10.1046/j. 1474-9728.2003.00060. x.

Gunta, S.S., Thadhani, R.I., Mak, R.H., 2013. The effect of vitamin D status on risk factors for cardiovascular disease. Nat. Rev. Nephrol. 9, 337–347, http://dx.doi.org/10.1038/nrneph.2013.74.

Hansen, M., Hsu, A.L., Dillin, A., Kenyon, C., 2005. New genes tied to endocrine, metabolic, and dietary regulation of lifespan from a Caenorhabditis elegans genomic RNAi screen. PLoS Genet. 1, 119–128, http://dx.doi.org/10.1371/journal.pgen.0010017.

Hjelmborg, J., Iachine, I., Skytthe, A., Vaupel, J.W., McGue, M., Koskenvuo, M., Kaprio, J., Pedersen, N.L., Christensen, K., 2006. Genetic influence on human lifespan and longevity. Hum. Genet. 119 (3), 312–321, http://dx.doi.org/10.1007/s00439-006-0144-y.

Kumsta, C., Ching, T.T., Nishimura, M., Davis, A.E., Gelino, S., Catan, H.H., Yu, X., Chu, C.C., Ong, B., Panowski, S.H., Baird, N., Bodmer, R., Hsu, A.L., Hansen, M., 2014. Integrin-linked kinase modulates longevity and thermotolerance in *C. elegans* through neuronal control of HSF-1. Aging Cell 13, 419–430, http://dx.doi.org/10.1111/acel.12189.

Kunnas, T., Nikkari, S.T., 2014. Contribution of syndecan-4 genetic variants to hypertension, the TAMRISK study. BMC Res. Notes 7, 815, http://dx.doi.org/10.1186/1756-0500-7-815.

Laaksi, I.T., Ruohola, J.P., Ylikomi, T.J., Auvinen, A., Haataja, R.I., Pihlajamäki, H.K., Tuohimaa, P.J., 2006. Vitamin D fortification as public health policy: significant improvement in vitamin D status in young Finnish men. Eur. J. Clin. Nutr. 60, 1035–1038, http://dx.doi.org/10.1038/sj.ejcn.1602414.

Lefebvre, P., Benomar, Y., Staels, B., 2010. Retinoid X receptors: common heterodimerization partners with distinct functions. Trends Endocrinol. Metab. 21, 676–683, http://dx.doi.org/10.1016/j.tem.2010.06.009.

McVean and the 1000 Genomes Project Consortium, 2012. An integrated map of genetic variation from 1092 human genomes. Nature 491, 56–65, http://dx.doi.org/10.1038/nature11632.

Miller, M., Stone, N.J., Ballantyne, C., Bittner, V., Criqui, M.H., Ginsberg, H.N., Goldberg, A.C., Howard, W.J., Jacobson, M.S., Kris-Etherton, P.M., Lennie, T.A., Levi, M., Mazzone, T., Pennathur, S., American, H., eart, A., ssociation, C., linical, L., ipidology, T., hrombosis, P., revention, C., the, C., on, N., utrition, P., hysical, A., ctivity, M., etabolism, C., on, A., rteriosclerosis, T., hrombosis, V., ascular, B., iology, C., on, C., ardiovascular, N., ursing, C., the, K., in, C., ardiovascular, D., 2011. Triglycerides and cardiovascular disease: a scientific statement from the American Heart Association. Circulation 123, 2292–2333, http://dx.doi.org/10.1161/CIR.0b013e3182160726.

Montesanto, A., Dato, S., Bellizzi, D., Rose, G., Passarino, G., 2012. Epidemiological, genetic and epigenetic aspects of the research on healthy ageing and longevity. Immun. Ageing 9 (April (1)), 6, http://dx.doi.org/10.1186/1742-4933-9-6.

Morgan, M.R., Humphries, M.J., Bass, M.D., 2007. Synergistic control of cell adhesion by integrins and syndecans. Nat. Rev. Mol. Cell Biol. 8, 957–969, http://dx.doi.org/10.1038/nrm2289.

Moskalev, A.A., Aliper, A.M., Smit-McBride, Z., Buzdin, A., Zhavoronkov, A., 2014. Genetics and epigenetics of aging and longevity. Cell Cycle 13, 1063–1077, http://dx.doi.org/10.4161/cc.28433.

Murabito, J.M., Yuan, R., Lunetta, K.L., 2012. The search for longevity and healthy aging genes: insights from epidemiological studies and samples of long-lived individuals. J. Gerontol. A Biol. Sci. Med. Sci. 67, 470–479, http://dx.doi.org/10.1093/gerona/gls089.

Nishimura, M., Kumsta, C., Kaushik, G., Diop, S.B., Ding, Y., Bisharat-Kernizan, J., Catan, H., Cammarato, A., Ross, R.S., Engler, A.J., Bodmer, R., Hansen, M., Ocorr, K., 2014. A dual role for integrin-linked kinase and beta1-integrin in modulating cardiac aging. Aging Cell 13, 431–440, http://dx.doi.org/10.1111/acel.12193.

Pai, J.K., Cahill, L.E., Hu, F.B., Rexrode, K.M., Manson, J.E., Rimm, E.B., 2013. Hemoglobin a1c is associated with increased risk of incident coronary heart disease among apparently healthy, nondiabetic men and women. J. Am. Heart Assoc. 2, e000077, http://dx.doi.org/10.1161/JAHA.112.000077.

Pike, J.W., Meyer, M.B., 2010. The vitamin D receptor: new paradigms for the regulation of gene expression by 1,25-dihydroxyvitamin D(3). Endocrinol. Metab. Clin. North Am. 39, 255–269, http://dx.doi.org/10.1016/j.ecl.2010.02.007.

Razzolini, R., Tarantini, G., Ossena, G., Favaretto, E., Bilato, C., Manzato, E., Dalla-Volta, S., Iliceto, S., 2007. Non-cardiovascular mortality, low-density lipoprotein cholesterol and statins: a meta-regression analysis. Cardiology 109, 110–116, http://dx.doi.org/10.1159/000105551.

Reizes, O., Goldberger, O., Smith, A.C., Xu, Z., Bernfield, M., Bickel, P.E., 2006. Insulin promotes shedding of syndecan ectodomains from 3T3-L1 adipocytes: a proposed mechanism for stabilization of extracellular lipoprotein lipase. Biochemistry 45 (18), 5703–5711, http://dx.doi.org/10.1021/bi052263h.

Schadt, E.E., Molony, C., Chudin, E., Hao, K., Yang, X., Lum, P.Y., Kasarskis, A., Zhang, B., Wang, S., Suver, C., Zhu, J., Millstein, J., Sieberts, S., Lamb, J., GuhaThakurta, D., Derry, J., Storey, J.D., Avila-Campillo, I., Kruger, M.J., Johnson, J.M., Rohl, C.A., van Nas, A., Mehrabian, M., Drake, T.A., Lusis, A.J., Smith, R.C., Guengerich, F.P., Strom, S.C., Schuetz, E., Rushmore, T.H., Ulrich, R., 2008. Mapping the genetic architecture of gene expression in human liver. PLoS Biol. 6, e107, http://dx.doi.org/10.1371/journal.pbio.0060107.

Schupf, N., Costa, R., Luchsinger, J., Tang, M.X., Lee, J.H., Mayeux, R., 2005. Relationship between plasma lipids and all-cause mortality in nondemented elderly. J. Am. Geriatr. Soc., 219–226, http://dx.doi.org/10.1111/j.

Sentinelli, F., Minicocci, I., Montali, A., Nanni, L., Romeo, S., Incani, M., Cavallo, M.G., Lenzi, A., Arca, M., Baroni, M.G., 2013. Association of RXR-gamma gene variants with familial combined hyperlipidemia: genotype and haplotype analysis. J. Lipids 2013, 517943, http://dx.doi.org/10.1155/2013/517943.

So, H.C., Sham, P.C., 2011. Robust association tests under different genetic models, allowing for binary or quantitative traits and covariates. Behav. Genet. 41, 768–775, http://dx.doi.org/10.1007/s10519-011-9450-9.

Tikhonoff, V., Casiglia, E., Mazza, A., Scarpa, R., Thijs, L., Pessina, A.C., Staessen, J.A., 2005. Low-density lipoprotein cholesterol and mortality in older people. J. Am. Geriatr. Soc. 53 (12), 2159–2164, http://dx.doi.org/10.1111/j.1532-5415.2005.00492.x.

Vimaleswaran, K.S., Cavadino, A., Berry, D.J., Mangino, M., Andrews, P., Moore, J.H., Spector, T.D., Power, C., Järvelin, M.R., Hyppönen, E., 2014. Interaction between allelic variations in vitamin D receptor and retinoid X receptor genes on metabolic traits. BMC Genet. 15, 37, http://dx.doi.org/10.1186/1471-2156-15-37.

Ward, L.D., Kellis, M.H., 2012. HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. Nucleic Acids Res 40, D930-4, http://dx.doi.org/10.1093/nar/gkr917.

Wigginton, J.E., Cutler, D.J., Abecasis, G.R., 2005. A note on exact tests of Hardy–Weinberg equilibrium. Am. J. Hum. Genet. 76, 887–893, http://dx.doi.org/10.1086/429864 .

Wilson, R.H., Lai, C.Q., Lyman, R.F., Mackay, T.F., 2013. Genomic response to selection for postponed senescence in *Drosophila*. Mech. Ageing Dev. 134, 79–88, http://dx.doi.org/10.1016/j.mad.2012.11.00.

Wolfson, M., Budovsky, A., Tacutu, R., Fraifeld, V., 2009. The signaling hubs at the crossroad of longevity and age-related disease networks. Int. J. Biochem. Cell Biol. 41, 516–520, http://dx.doi.org/10.1016/j.biocel.2008.08.026.

Xian, X., Gopal, S., Couchman, J.R., 2010. Syndecans as receptors and organizers of the extracellular matrix. Cell Tissue Res. 339, 31–46, http://dx.doi.org/10.1007/s00441-009-0829-3.