Common *SIRT1* variants modify the effect of abdominal adipose tissue on aging-related lung function decline

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Abstract Lung function is an independent predictor of mortality and serves as an aging marker in never smokers. The protein sirtuin-1 of gene *SIRT1* has profound anti-inflammatory effects and regulates metabolic pathways. Its suggested longevity effects on lower organisms remain poorly studied in humans. In 1132 never smokers of the population-based SAPALDIA cohort, we investigated associations between single nucleotide polymorphisms (SNPs; *rs730821*, *rs10997868*, *rs10823116*) of *SIRT1* and aging-related lung function decline over 11 years in terms of change in forced

expiratory volume in the first second (FEV₁), forced vital capacity (FVC), FEV₁/FVC ratio, and forced expiratory flow between 25 and 75 % of FVC (FEF_{25–75}) using multiple linear regression models. Interactions between the *SIRT1* SNPs and adiposity parameters (body mass index (BMI), its change and weight gain) were tested by including multiplicative interaction terms into the models. *SIRT1* polymorphisms exhibited no main effects, but modified the association between obesity measures and FEV₁/FVC and FEF_{25–75} decline ($p = 0.009–0.046$). Per risk allele, FEV₁/FVC decline

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was accelerated up to -0.5% (95 % CI -1.0 to 0%) and -0.7% (-1.3 to -0.2%) over interquartile range increases in BMI (2.4 kg/m^2) or weight (6.5 kg), respectively. For FEF_{25–75} decline, corresponding estimates were -57 mL/s (-117 to 4 mL/s) and -76 mL/s (-1429 to -9 mL/s). Interactions were not present in participants with genetically lowered C-reactive protein concentrations. Genetic variation in *SIRT1* might therefore affect lung function and human longevity by modifying subclinical inflammation arising from abdominal adipose tissue.

Keywords Sirtuin-1 · Lung · Metabolism · Inflammation · Cohort study · Population-based

Introduction

The level of lung function is one of the most consistent and long-known predictors of survival (Burney and Hooper 2011; Knuiman et al. 1999; Schunemann et al. 2000). Lung function naturally starts to decline at the age of 30 years (Quanjer et al. 2012), and accelerated decline in adult life was repeatedly associated with increased mortality, not only due to cardiovascular and respiratory disease (Tockman et al. 1995) which could be attributable to smoking but also with all-cause mortality and in never smokers (Mannino et al. 2006; Ryan et al. 1999). According to recent findings from the British Whitehall study, the direct effects of smoking only explain 4.9 % of the association between lung function and mortality, whereas 21.3 % could be attributed to inflammatory processes in the body and a further 9.8 % to lifestyle factors including nutrition and body fitness (Sabia et al. 2010). Moreover, increases in inflammatory biomarkers were also longitudinally associated with accelerated lung function decline (Ahmadi-Abhari et al. 2014; Shaaban et al. 2006). The level of lung function thus not only reflects past pulmonary exposures to tobacco smoke or environmental hazards or presence of respiratory disease but also equally functions as a biomarker of aging processes in the body (Albrecht et al. 2014).

Biological processes related to aging include persistent low-grade inflammation, mitochondrial malfunctioning with reduced energy expenditure, reduced nutrient sensing, and metabolic disturbances (Franceschi et al. 2000; Lopez-Otin et al. 2013). The accumulation of intra-abdominal adipose tissue during normal aging (Picard and Guarente 2005) plays an important role in this respect, as it alters the endocrine

profile of adipocytes towards inflammation, dyslipidemia, and insulin resistance (Berg and Scherer 2005; Calabro et al. 2009). Increased abdominal adiposity is associated with adverse cardiovascular outcomes (Berg and Scherer 2005) and was also repeatedly related to impaired lung function (Leone et al. 2009; Ochs-Balcom et al. 2006; Wehrmeister et al. 2012). In studies relying on waist-to-hip ratio, waist circumference, or imaging techniques as markers of intra-abdominal fat tissue, higher abdominal fat mass was most often associated with increased overall mortality (Bigaard et al. 2005; Reis et al. 2009; Zhang et al. 2008).

A central protein involved in the regulation of aging-related metabolic functions is sirtuin-1, which is encoded by the gene *SIRT1*. Sirtuin-1 is a class III histone deacetylase regulating nuclear transcription. It was first described in yeast *Saccharomyces cerevisiae* and suggested to extend lifespan under starving conditions (Burnett et al. 2011; Guarente 2011; Herranz et al. 2010; Herranz and Serrano 2010; Kennedy et al. 1997; Rizki et al. 2011; Viswanathan and Guarente 2011). Upregulation of sirtuin-1 reduces lipid storage, mobilizes fatty acids in white adipose tissue, and increases their β -oxidation in liver and muscle tissue. It activates biosynthesis of mitochondria and increases peripheral insulin sensitivity and central nutrient sensing in the hypothalamus (Guarente 2011). By modulating the expression of the cardinal pathways of forkhead transcription factors of class O (FoxOs), nuclear factor-kappa-B (NF κ B), and p53, sirtuin-1 also plays a major role in cellular defense against oxidative stress and inflammation, and at higher stress levels, it protects cells from senescence and apoptosis (Chong et al. 2012; Radak et al. 2013). Likewise, experimental studies in rodents have shown that *SIRT1* overexpression mitigates matrix metalloprotease imbalance, cell senescence, and autophagy in lungs upon cigarette smoke exposure (Hwang et al. 2010; Yao et al. 2013; Yao et al. 2014).

Because of the proposed longevity effects in several species, sirtuin-1 and other members of its enzyme family have been in the focus of aging research over recent years. Studies in humans have thereby focused on cardiovascular risk factors and health outcomes due to the anti-inflammatory and profound metabolic functions of sirtuin-1. Population-based cohort studies found significant associations between common single nucleotide polymorphisms (SNPs) in *SIRT1* and increased glucose tolerance, body mass index, overweight, and atherosclerosis (Figarska et al. 2013; Kedenko et al. 2014;

Zillikens et al. 2009a), but except for one study (Figarska et al. 2013), not with survival (Flachsbart et al. 2006; Kuningas et al. 2007; Zillikens et al. 2009b). Moreover, a Dutch study using data from two population-based cohorts assessed the relationship of *SIRT1* SNPs with lung function levels but did not observe significant associations (Siedlinski et al. 2012). However, the diverse regulatory roles of sirtuin-1 in the body also warrant consideration of several pathways and potential interactions.

In the current study, we investigated age-related lung function decline in never smokers as a proxy for aging processes. We hypothesized that the level of *SIRT1* expression could affect the course of lung function decline in different ways, as depicted in Fig. 1: first, high sirtuin-1 concentrations could boost anti-inflammatory and anti-oxidative defenses locally in the lungs and protect them from inflammation, apoptosis, and tissue remodeling (path A); second, it could reduce the amount of abdominal fat tissue and consequently the accompanying, subclinical inflammation (path B); third, sirtuin-1 could mitigate the systemic, inflammatory effects arising from abdominal fat accumulation (path C).

Using data from the population-based Swiss SAPALDIA cohort study, we thus investigated possible associations and interactions between lung function decline, common SNPs in *SIRT1*, and several measures of body fat mass (including body mass index (BMI), its change and gain in weight) as potential evidence for the involvement of *SIRT1* in human aging.

Methods

Design and study population

The Swiss cohort study on air pollution and lung and heart disease in adults (SAPALDIA) recruited adults aged 18–60 years from eight Swiss communities in 1991 and followed them up in the year 2002. A detailed overview of the study design and methods is given elsewhere (Ackermann-Lieblich et al. 2005). The current analysis comprised a study sample (Fig. 2) restricted to 1132 non-asthmatic, never smoking adults who attended the baseline and the first follow-up examination including spirometry, who had genotype data on common *SIRT1* variants available, and who were aged at least 30 years at baseline. The restrictions were applied since our aim was to study the association of *SIRT1* genetic variation with natural lung function decline as a marker of biological aging. Asthmatic participants were also excluded due to their known, differing genetic background affecting lung function (Imboden et al. 2012). Current smokers at follow-up were included in a sensitivity analysis to validate the functional impact of genetic associations.

All study participants gave written informed consent, and the study was approved by the Swiss Academy of Medical Sciences as well as the respective regional ethics committees.

Fig. 1 Hypothesized paths of sirtuin-1 effects on lung function decline as proxy measure of aging

Data collection

Spirometry

Spirometry was done with Sensormedics devices (Sensormedics model 2200, Yorba Linda, USA) following the protocol of the European Community Respiratory Health Survey. Devices were calibrated daily, and comparability between devices was checked. Participants performed three to eight forced expiratory maneuvers which were subjected to American Thoracic Society quality criteria (American Thoracic Society 1987). For the forced expiratory volume in the first second of exhalation (FEV₁) and forced vital capacity (FVC), at least two acceptable values were recorded. The FEV₁/FVC ratio (a marker of airway obstruction) and mean forced expiratory flow between 25 and 75 % of FVC (FEF_{25–75}) were derived from the maneuver with the highest sum of FVC and FEV₁. Longitudinal change was calculated as the difference between baseline and follow-up value. SAPALDIA-specific lung function equations were used for the calculation of percent predicted lung function values and lower limits of normal (Brandli et al. 2000).

Body measures

For the measurement of weight and height, participants wore no shoes or heavy clothes. BMI was calculated by dividing weight (in kilograms) by the square of height (in meters). Change in BMI and weight was calculated as the respective difference between follow-up and baseline value. Overweight was defined as BMI $\geq$ 75th percentile of the observed population distribution (corresponding to BMI $\geq$ 26.3 kg/m²). This definition was chosen because more suitable measures such as waist-to-hip ratio were not available. Moreover, the optimal BMI threshold to capture subclinical inflammation is unknown, and previous studies have shown that the WHO overweight definition (BMI $\geq$ 25 kg/m²) does not correlate well with the amount of abdominal adipose tissue (Goyal et al. 2014). Weight gain during 11 years of follow-up was used to approximate the increase in abdominal adipose tissue in our adult population. Given our interest in gain of abdominal adipose tissue, participants experiencing a weight loss of 2 kg (corresponding to the median of the observed weight loss distribution) or more were excluded from weight gain analyses.

Covariates

Questionnaires were applied to gather information on smoking behavior and socioeconomic factors. Education was categorized into low (primary school only), medium (high school), and high (university or college degree) depending on the highest attained degree at follow-up.

Never smokers were defined as having smoked less than 20 packs of cigarettes or 360 g of tobacco during their lifetime. Current smokers indicated active smoking within 30 days prior to the follow-up examination.

SIRT1 genotypes

Blood samples were collected at follow-up examination. Genomic DNA was extracted manually from EDTA-buffered whole blood using the Puregene™ DNA Isolation Kit (Gentra Systems, Plymouth, MN, USA).

The genotype data on *SIRT1* SNPs used in this analysis consisted of three tagging SNPs (*rs730821*, *rs10997868*, *rs10823116*) obtained from two different genotyping platforms (Fig. 2). The three SNPs were selected to represent the common genetic variation in our study population in terms of the observed linkage disequilibrium in the *SIRT1* region.

Genotype data A was typed on the Illumina Human 610Kquad BeadChip (Moffatt et al. 2010) and subsequently imputed to 2.5 Mio SNPs using the HapMap v22 CEPH reference panel of Utah residents with ancestry from northern and western Europe (International HapMap Consortium 2005) to fill in missing genotype values and improve genomic coverage. Genotype data B was typed on the Illumina Human OmniExpress-Exome BeadChip in a non-overlapping sample of the SAPALDIA population. Stringent quality control filters were applied to both platforms.

Details of the genotyping procedures and biomarker measurements are given in Online Resource 1.

Statistical analysis

The distributions of sex, age, educational level, lung function parameters FEV₁, FVC, FEV₁/FVC, FEF_{25–75}, and their change during the first 11 years of follow-up, as well as those of adiposity measures and CRP levels, were tabulated for the whole study population and for normal and overweight subgroups.

Fig. 2 Selection of study sample

Main effects of *SIRT1* SNPs were modeled using multiple linear regression of change in FEV₁, FVC, FEV₁/FVC, and FEF_{25–75} adjusting for the percent predicted lung function value at baseline, sex, age, height, education, and study area. Age and height were centered at the median value of the population distribution for all regression analyses. SNPs were modeled under an additive genetic model. To account for uncertainty in imputation, observations with imputed *SIRT1* genotypes (genotypes from genotyping platform A) were weighted with the imputation quality metric in regression analysis. Effect estimates were compared across models incrementally adjusting for BMI at baseline (centered at its median), its change, or weight change during follow-up.

Next, interaction analyses were performed by including multiplicative interaction terms between *SIRT1* SNPs and change in BMI or weight gain during follow-up in multiple regression models adjusting for the same set of covariates. Estimates were scaled to represent effects over an increase of interquartile range (IQR) in BMI (2.42 units) and weight (6.5 kg). For weight gain, interaction analyses were additionally adjusted for baseline BMI.

To evaluate the possible impact of multiple testing on our results, interaction models with change in BMI and weight gain were re-specified as multivariate multiple regressions on all four outcomes (thereby taking account of the correlation between the lung function decline

parameters). Interaction terms and main effects of all three SNPs were simultaneously included into the model, and the null hypothesis that none of the SNPs presented a statistically significant interaction was eventually tested using a joint Wald test on all interaction terms.

The interaction models were stratified by the genotypes of C-reactive protein (*CRP*) gene SNP *rs1800947* to assess the influence of inflammatory pathways. *rs1800947* is a functional SNP whose C-alleles have been associated with lower systemic levels of C-reactive protein (Kettunen et al. 2011). Due to the low minor allele frequency of 6.4 %, the rare allele genotypes *GC* and *CC* of *rs1800947* were grouped together and compared to the common *GG* genotype.

To exclude undiagnosed cases of lung disease, interaction models were recalculated after excluding participants with spirometric obstruction at baseline ($FEV_1/FVC < 5$ th percentile of the population-specific distribution in never smokers, representing the lower limit of normal) or with wheezing without a cold in 12 months before examination in a sensitivity analysis.

Finally, *SIRT1* SNP effects were reassessed in current smokers at follow-up regarding size and direction of genetic main effects on lung function to validate their functional impact in comparison to never smokers.

All analyses were conducted using STATA IC version 12.1 (StataCorp, College Station, TX, USA). Two-sided α -values of 0.05 were chosen as significance thresholds for main effects and interactions, respectively. Multivariate analyses were done using STATA module “mvreg.”

Results

Characteristics of study population

The basic characteristics of the whole study population and of normal and overweight subgroups are shown in Table 1. 60.7 % of our study population was female, and 12.3 % had only primary school education. Mean (standard deviation) age was 44.8 (8.7) years. Baseline weight was 67.4 (11.9) kilograms (kg) and BMI 23.8 (3.5) kg/meter² (kg/m²). Mean lung function values were 3.44 (0.78) liters (L), 4.38 (1.00) L, 78.8 (6.7) %, and 3.27 (1.11) L/second (L/s) for FEV_1 , FVC, FEV_1/FVC , and FEF_{25-75} , respectively. These values declined by 0.38 (0.29) L, 0.30 (0.39) L, 3.7 (5.0) %, and 0.73

(0.67) L/s over 11 years of follow-up. During the same period, participants gained 6.1 (5.1) kg weight on average, and their BMI increased by 2.2 (2.0) units. Participants with overweight at baseline were on average older, had less education, and slightly worse FEV_1/FVC and FEF_{25-75} values than normal weight participants. Of note, overweight participants had considerably higher C-reactive protein levels (median 1.7, IQR 0.9–3.7 mg/L) than those with normal weight (0.8, IQR 0.4–1.7 mg/L).

Main effects of *SIRT1* SNPs on lung function decline

No statistically significant associations of *SIRT1* SNPs *rs730821*, *rs10997868*, and *rs10823116* with change in lung function parameters were observed, and additional adjustment for baseline BMI value, change in BMI or weight during follow-up only slightly altered the effect estimates (Table 1 in Online Resource 2).

Main effects of *SIRT1* SNPs stratified by overweight at baseline

Within strata of normal weight and overweight, no statistically significant main effects were observed (Table 2). However, allele effects on change in FEV_1/FVC differed significantly between the strata for SNP *rs10823116*: 0.2 % (95 % confidence interval −0.6 to 1.1) in normal weight versus −1.8 % (−3.9 to 0.3) in overweight participants ($p = 0.048$ for interaction). Effect estimates also differed significantly for change in FEF_{25-75} ($p = 0.022$ for interaction) and amounted to 47 mL/s (−57 to 150) in normal weight versus −244 mL/s (−495 to 6) in overweight participants. No interactions were observed between *SIRT1* SNPs and baseline BMI as continuous variable (data not shown).

Interaction effects with change in BMI and weight

SNP *rs730821* presented a significant interaction with change in BMI on the change in FEV_1/FVC ratio, while corresponding estimates were marginally significant for *rs10997868* and *rs10823116* ($p = 0.033$ to $p = 0.093$; Table 3). Over an IQR increase in BMI, FEV_1/FVC decline was accelerated by −0.5 % (−1.0 to 0.0; *rs730821*) per each effect allele compared to the homozygous reference group. The corresponding estimate for an IQR increase in weight was larger, amounting to −0.7 % (−1.3 to −0.2; *rs730821*), and interaction p

Table 1 Baseline characteristics of whole study population and by strata of overweight

Variable	All			Normal weight			Overweight		
	<i>n</i>	Estimates		<i>n</i>	Estimates		<i>n</i>	Estimates	
Female sex	1132	687	/60.7	858	545	/63.5	274	142	/51.8
Baseline age (years)	1132	44.8	(8.7)	858	43.9	(8.5)	274	47.5	(8.7)
Education primary only	1132	139	/12.3	858	83	/9.7	274	56	/20.4
High school	1132	782	/69.1	858	599	/69.8	274	183	/66.8
College/university	1132	211	/18.6	858	176	/20.5	274	35	/12.8
Baseline FEV ₁ (L)	1132	3.44	(0.78)	858	3.45	(0.76)	274	3.41	(0.86)
FVC (L)	1132	4.38	(1.00)	858	4.37	(0.95)	274	4.39	(1.12)
FEV ₁ /FVC (%)	1132	78.8	(6.7)	858	79.1	(6.8)	274	77.9	(6.2)
FEF _{25–75} (L/s)	1132	3.27	(1.11)	858	3.30	(1.11)	274	3.16	(1.10)
Change in FEV ₁ (L)	1132	-0.38	(0.29)	858	-0.37	(0.29)	274	-0.42	(0.31)
FVC (L)	1132	-0.30	(0.39)	858	-0.27	(0.38)	274	-0.39	(0.41)
FEV ₁ /FVC (%)	1132	-3.7	(5.0)	858	-3.9	(4.8)	274	-3.1	(5.3)
FEF _{25–75} (L/s)	1132	-0.73	(0.67)	858	-0.74	(0.64)	274	-0.69	(0.75)
Baseline height (cm)	1132	168	(9)	858	168	(9)	274	167	(10)
Baseline weight (kg)	1132	67.4	(11.9)	858	63.3	(9.2)	274	80.2	(10.5)
Change in weight (kg) ^a	1071	6.1	(5.1)	824	5.7	(4.9)	247	7.3	(5.7)
Baseline BMI (kg/m ²)	1132	23.8	(3.5)	858	22.3	(2.1)	274	28.6	(2.5)
Change in BMI (kg/m ²)	1132	2.2	(2.0)	858	2.1	(1.9)	274	2.4	(2.5)
Follow-up CRP (mg/L)	1095	1	(0.5/2.1)	842	0.8	(0.4/1.7)	253	1.7	(0.9/3.7)

Estimates are in numbers/% for count data, mean (standard deviation) for continuous data, and median (25th/75th percentile) for CRP
 CRP C-reactive protein serum value, values ≥ 10 mg/L have been excluded, FEV₁ forced expiratory volume in 1 s of expiration, FVC forced vital capacity, FEF_{25–75} forced expiratory flow between 25th and 75th percentile of FVC, L liters, s second

^a Values ≤ -2 kg (the median value of all participants with weight loss) have been excluded

values were stronger ($p = 0.009$, *rs730821*; $p = 0.070$, *rs10997868*; and $p = 0.094$, *rs10823116*).

Similar interaction patterns were observed on change in FEF_{25–75}: interactions with an IQR increase in BMI were marginally significant ($p = 0.66$ to $p = 0.10$ for interaction), and FEF_{25–75} decline was accelerated up to -95 mL/s (-204 to 13 ; *rs10823116*) per each effect allele. Significant interactions with an IQR increase in weight were observed for SNPs *rs730821* and *rs10997868* ($p = 0.025$ and $p = 0.046$, respectively), and corresponding interaction estimates were -76 mL/s (-142 to -9 ; *rs730821*) and -56 mL/s (-111 to -1 ; *rs10997868*).

In multivariate multiple regression models, the joined Wald test of the null hypothesis that none of the SNPs presented a statistically significant interaction yielded p values of $p = 0.086$ and $p = 0.032$ for the model with change in BMI and weight gain, respectively.

Interaction analyses stratified by CRP *rs1800947* genotype

When stratifying the interaction analyses by GC/CC versus GG genotype of CRP SNP *rs1008947*, the interaction effects of *SIRT1* variation only persisted on the common GG stratum (Table 4). The GG stratum had 0.2 mg/L higher average C-reactive protein levels than the GC/CC stratum within the normal serum range up to 10 mg/L ($p = 0.060$ for the Mann-Whitney test of difference in medians).

Sensitivity analyses

Excluding participants with obstructive lung function at baseline or wheezing without a cold at either examination did not substantially alter the observed interaction patterns (Tables 2 and 3 in Online Resource 2).

Table 2 *SIRT1* SNP main effects on change in lung function stratified by overweight status

Outcome	SNP	Eff. all.	Freq	Normal weight (<i>n</i> = 858)		Overweight (<i>n</i> = 274)		All (<i>n</i> = 1132)	
				β -coeff. (95 % CI)	<i>p</i>	β -coeff. (95 % CI)	<i>p</i>	β -coeff. (95 % CI)	<i>p</i>
Change in FEV ₁ (in mL)	<i>rs730821</i> ^a	C	0.3	3 (−27 to 34)	0.83	−11 (−75 to 53)	0.74	0 (−28 to 27)	0.98
	<i>rs10997868</i>	C	0.68	−11 (−35 to 14)	0.41	−21 (−77 to 35)	0.46	−12 (−35 to 11)	0.30
	<i>rs10823116</i>	G	0.13	−20 (−73 to 33)	0.46	−117 (−242 to 9)	0.068	−38 (−88 to 12)	0.13
Change in FVC (in mL)	<i>rs730821</i> ^a	C	0.38	0 (−43 to 44)	0.99	−4 (−85 to 78)	0.93	1 (−38 to 39)	0.97
	<i>rs10997868</i>	C	0.68	−13 (−49 to 23)	0.48	−33 (−106 to 41)	0.38	−14 (−46 to 19)	0.41
	<i>rs10823116</i>	G	0.13	−39.2 (−106 to 28)	0.25	−84 (−248 to 80)	0.32	−42 (−105 to 22)	0.20
Change in FEV ₁ /FVC (in percent)	<i>rs730821</i> ^a	C	0.38	0.1 (−0.5 to 0.6)	0.80	−0.2 (−1.2 to 0.9)	0.75	0.0 (−0.5 to 0.4)	0.85
	<i>rs10997868</i>	C	0.68	0.0 (−0.4 to 0.4)	0.93	0.1 (−0.8 to 1.0)	0.84	0.0 (−0.4 to 0.3)	0.85
	<i>rs10823116</i>	G	0.13	0.2 (−0.6 to 1.1)*	0.64	−1.8 (−3.9 to 0.3)*	0.099	−0.3 (−1.1 to 0.5)	0.48
Change in FEF _{25–75} (in mL/s)	<i>rs730821</i> ^a	C	0.38	9 (−52 to 71)	0.77	−19 (−156 to 118)	0.79	−4 (−61 to 52)	0.88
	<i>rs10997868</i>	C	0.68	−13 (−62 to 37)	0.62	−10 (−128 to 108)	0.87	−18 (−65 to 28)	0.45
	<i>rs10823116</i>	G	0.13	47 (−57 to 150)*	0.37	−244 (−495 to 6)*	0.056	−20 (−117 to 78)	0.69

Estimates are given per effect allele. Negative values mean an acceleration and positive an attenuation of lung function decline. All models were adjusted for percent predicted baseline value of lung function, sex, age (centered to median), height (centered to median), educational level, and study area

FEV₁ forced expiratory volume in 1 s of expiration, FVC forced vital capacity, FEF_{25–75} forced expiratory flow between 25th and 75th percentile of FVC, overweight BMI $\geq$ 75th percentile of the observed distribution, Eff. all. effect allele, Freq effect allele frequency, β -coeff beta-coefficient, 95 % CI 95 % confidence interval, *p* *p* value

*Statistically significant interaction (*p* < 0.05)

^a *n* = 1130 in whole study sample and *n* = 856 in normal weight due to missing genotype data

SIRT1 SNP effects on smokers

In current smokers at follow-up, significant main effects were observed for SNP *rs730821*, accelerating the decline in FEV₁ by −59 mL (−106 to −13) per each effect allele and in FVC by −77 mL (−135 to −18) (Table 4 in Online Resource 2). A marginally significant main effect was observed for *rs10997868* (*p* = 0.051) with an estimated accelerated FVC decline of −46 mL (−93 to 0) per allele.

Discussion

Using data from a general population sample of non-smoking, non-asthmatic adults aged 30 years and older, the present study investigated long-term effects of genetic variation in *SIRT1* on the age-related decline in lung function as a marker of biological aging. *SIRT1* SNPs *rs730821*, *rs10997868*, and *rs10823116* were not associated directly with lung function decline, and adjustments for body weight measures hardly affected the respective main effect estimates. However, SNPs

rs730821 and *rs10997868* showed significant evidence of interaction with increases in BMI or weight, while for *rs10823116*, estimates were marginally significant. The interaction was restricted to participants with normal genetic regulation of C-reactive protein serum levels.

The absence of genetic main effects on our never smoking study population is in line with previous findings from a Dutch population-based study, where no significant associations between *SIRT1* tagging SNPs and lung function level were observed (but partial evidence was found for interactions with smoking) (Siedlinski et al. 2012). In terms of our hypothesized pathways of sirtuin-1 effects, our results do not support direct effects on oxidative stress defense in the lungs of never smokers (corresponding to path A in Fig. 1). However, the observed significant genetic main effects on smokers point to a potential, protective role in high-level oxidative stress exposures. Estimates for SNP main effects on lung function decline were only slightly affected by the inclusion of body weight measures. Regulation of body fat mass by sirtuin-1 (corresponding to path B in Fig. 1) is thus less likely to underlie effects on lung function decline. The observed interactions between *SIRT1* SNPs and increased body weight measures rather

Table 3 Interaction effect estimates between *SIRT1* SNPs and change in BMI or weight on change in lung function

Outcome	SNP	Eff. all.	Freq	Effect estimates for interaction with					
				change in BMI (<i>n</i> = 1132)			change in weight ^b (<i>n</i> = 1071)		
				β -coeff.	95 % CI	<i>p</i>	β -coeff	95 % CI	<i>p</i>
Change in FEV ₁ (in mL)	<i>rs730821</i> ^a	C	0.38	7	(-27 to 41)	0.68	5	(-33 to 42)	0.81
	<i>rs10997868</i>	C	0.68	-3	(-29 to 23)	0.82	-10	(-38 to 18)	0.47
	<i>rs10823116</i>	G	0.13	-33	(-95 to 29)	0.29	-41	(-111 to 29)	0.25
Change in FVC (in mL)	<i>rs730821</i> ^a	C	0.38	43	(-7 to 92)	0.091	49	(-7 to 105)	0.087
	<i>rs10997868</i>	C	0.68	18	(-21 to 56)	0.36	11	(-29 to 51)	0.60
	<i>rs10823116</i>	G	0.13	-7	(-83 to 70)	0.87	-10	(-97 to 77)	0.82
Change in FEV ₁ /FVC (in percent)	<i>rs730821</i> ^a	C	0.38	-0.5	(-1.0 to 0.0)	0.033*	-0.7	(-1.3 to -0.2)	0.009*
	<i>rs10997868</i>	C	0.68	-0.4	(-0.8 to 0.1)	0.085	-0.4	(-0.9 to 0.0)	0.070
	<i>rs10823116</i>	G	0.13	-0.8	(-1.6 to 0.1)	0.093	-0.9	(-1.9 to 0.2)	0.094
Change in FEF ₂₅₋₇₅ (in mL/s)	<i>rs730821</i> ^a	C	0.38	-57	(-117 to 4)	0.066	-76	(-142 to -9)	0.025*
	<i>rs10997868</i>	C	0.68	-41	(-90 to 9)	0.10	-56	(-111 to -1)	0.046*
	<i>rs10823116</i>	G	0.13	-95	(-204 to 13)	0.086	-112	(-239 to 15)	0.084

Estimates for the multiplicative interaction term are given per effect allele and interquartile range increase in BMI (2.42 units) and weight (6.5 kg). Negative values mean an acceleration and positive an attenuation of lung function decline. All models were adjusted for percent predicted baseline value of lung function, sex, age (centered to median), height (centered to median), educational level, and study area

FEV₁ forced expiratory volume in 1 s of expiration, FVC forced vital capacity, FEF₂₅₋₇₅ forced expiratory flow between 25th and 75th percentile of FVC, BMI body mass index, Eff. all. effect allele, Freq effect allele frequency, β -coeff beta-coefficient, 95 % CI 95 % confidence interval, *p* *p* value of interaction

*Statistically significant interaction (*p* < 0.05)

^a *n* = 1130 for change in BMI and *n* = 1069 for weight change analyses due to missing genotype data

^b For analyses with change in weight, participants with change ≤ -2 kg (median of observed weight loss distribution) were excluded

suggest that differences in *SIRT1* expression modify the deleterious effects of abdominal adipose tissue on lung function decline (path C in Fig. 1). This effect modification has to the best of our knowledge not been described before. The patterns of interaction suggest that sirtuin-1 might modulate systemic low-grade inflammation arising from increased abdominal fat tissue. BMI only insufficiently captures the amount of central body fat relevant for subclinical inflammation (Goyal et al. 2014). Accordingly, we did not observe interaction effects with baseline BMI centered at 23.5 kg/m², but the interaction became notable in subjects with a BMI > 26.3 kg/m² (associated with higher serum C-reactive protein concentrations). Furthermore, stronger *SIRT1* interaction effects were observed with change in BMI and particularly with weight gain, which more likely reflect increases in abdominal fat tissue in adult populations. Finally, the interaction effects were restricted to participants with normal genetic *CRP* expression.

Limitations of our study were that only proxy measures of visceral fat mass were available. Moreover, the genetic effects under study were small and comprehensive assessment of potential interactions required multiple tests to be performed, all factors affecting the statistical power of our analysis. Nevertheless, the joined tests performed in multivariate, multiple regression models provide evidence that some of the observed SNP interactions are true findings. Furthermore, our definitions of overweight at baseline, BMI increase, and weight gain were strongly and positively associated with higher C-reactive protein levels at follow-up (all *p* < 0.0001, data not shown) and thus captured subclinical inflammatory states. Further, the functional impacts of the studied SNPs are not exactly known, and depending on the position, the numerous biological functions of sirtuin-1 can differently be affected. The variants were selected to represent common genetic variation in the Swiss general population, and all three were in non-coding gene

Table 4 Interaction effect estimates of *SIRT1* SNPs with change in BMI and weight gain stratified by *CRP* SNP *rs1800947*

Outcome	SNP	Effect allele	Freq	<i>CRP</i> SNP <i>rs1800947</i>					
				Wild type <i>GG</i> (<i>n</i> = 964 ^b)			Mutant alleles <i>GC/CC</i> (<i>n</i> = 137 ^b)		
				β-coeff.	95 % CI	<i>p</i>	β-coeff.	95 % CI	<i>p</i>
Interaction effect estimates for change in BMI									
Change in FEV ₁ /FVC	<i>rs730821</i> ^a	C	0.38	-0.7	(−1.2 to −0.2)	0.012*	0.3	−1.5 to 2.1	0.76
	<i>rs10997868</i>	C	0.68	-0.4	(−0.8 to 0.1)	0.12	-0.5	−1.8 to 0.8	0.45
	<i>rs10823116</i>	G	0.13	-0.7	(−1.6 to 0.3)	0.19	-2.4	−4.4 to −0.4	0.021*
change in FEF _{25–75} (in L/s)	<i>rs730821</i> ^a	C	0.38	-73	(−138 to −9)	0.025*	62	−149 to 273	0.57
	<i>rs10997868</i>	C	0.68	-43	(−96 to 11)	0.12	-22	−191 to 146	0.80
	<i>rs10823116</i>	G	0.13	-100	(−219 to 19)	0.099	-74	−292 to 145	0.51
Interaction effect estimates for weight gain									
Change in FEV ₁ /FVC (in percent)	<i>rs730821</i> ^a	C	0.38	-0.9	(−1.5 to −0.3)	0.003*	0.4	−1.1 to 1.9	0.62
	<i>rs10997868</i>	C	0.68	-0.5	(−1.0 to 0.1)	0.076	-0.5	−1.7 to 0.8	0.47
	<i>rs10823116</i>	G	0.13	-0.8	(−1.9 to 0.4)	0.19	-2.3	−4.5 to 0.0	0.054
Change in FEF _{25–75} (in mL/s)	<i>rs730821</i> ^a	C	0.38	-94	(−165 to −24)	0.009*	96	−114 to 306	0.37
	<i>rs10997868</i>	C	0.68	-63	(−123 to −4)	0.037*	7	−166 to 180	0.94
	<i>rs10823116</i>	G	0.13	-111	(−252 to 31)	0.13	-89	−357 to 179	0.52

Estimates for the interaction term are given per effect allele and interquartile range increase in BMI (2.42 units) and weight (6.5 kg). Negative values mean an acceleration and positive an attenuation of lung function decline. All models were adjusted for percent predicted baseline value of lung function, sex, age (centered to median), height (centered to median), educational level, and study area

FEV₁ forced expiratory volume in 1 s of expiration, FVC forced vital capacity, FEF₂₅₋₇₅ forced expiratory flow between 25th and 75th percentile of FVC, BMI body mass index, Freq effect allele frequency, β -coeff. beta-coefficient, 95 % CI 95 % confidence interval, *p* *p* value of interaction

*Statistically significant interaction (*p* < 0.05)

^a *n* = 963 in wild type stratum due to missing genotype data

^b *n* = 911 for wild type and *n* = 131 for mutant stratum after excluding participants with weight change ≤ -2 kg

regions. Queries in the HaploReg database yielded evidence from different, independent studies that SNPs *rs730821* and *rs10997868* are associated with *SIRT1* expression in blood (GTEx Consortium. Ardlie KG et al. 2015), and *rs10823116* is located in a DNase-sensitive site in lung cells (Ward and Kellis 2012). Further, all three SNPs tag several variants that potentially affect *SIRT1* expression via enhancer and promoter histone marks in lung and adipose tissue. Thus, our studied SNPs might directly affect gene expression or likely tag such regulatory variants in the region. Finally, the main results in our never smoking population were observed with decline in FEF₂₅₋₇₅ and not FEV₁ or FVC, which were the classical correlates of future mortality in previous studies. Due to their large surface area, the small airways are known to be an important compartment of early respiratory disease processes (Burgel

et al. 2011; Hamid 2012) were subclinical changes often manifest first. Accordingly, in our study sample of never smokers from the general population, inflammation-related aging processes presented most strongly in FEF₂₅₋₇₅ decline.

Strengths of our study include the population-based design and the availability of high-quality spirometry and genome-wide genotyping data for a large part of our cohort participants. This enabled us to study aging processes in a representative group of healthy, non-smoking adults using a lung function-based model of aging.

In conclusion, our study observed that genetic variation in *SIRT1* affects age-related lung function decline in never smoking adults from the general population by modifying the deleterious effects of weight gain, likely related to systemic low-grade inflammation from visceral adipose tissue. These results suggest that differences

in *SIRT1* expression might play a role in human aging via modulation of adipose tissue-related inflammation. In light of the increasing obesity epidemic and the availability of pharmacological substances and dietary constituents that affect the biological availability and activity of sirtuin-1, these findings could be relevant to public health considerations.

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