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Serum bilirubin is associated with lung function in a Swiss general population sample

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Serum bilirubin is associated with FEV_1/FVC and FEF_{25-75} in persons with low-grade inflammatory states from the general population.

ABSTRACT

Introduction

Bilirubin is a strong antioxidant. Increased serum levels were associated with respiratory disease and mortality risk. We studied the association of bilirubin with lung function in the SAPALDIA cohort.

Methods

Associations between natural logarithmized bilirubin and forced expiratory volume in the first second (FEV_1), forced vital capacity (FVC), FEV_1/FVC, and mean forced expiratory flow between 25%-75% of FVC (FEF_{25-75}) were tested using multiple linear regression in the whole study population (n=4195) and strata of ever smoking and high body mass index (BMI, defined by the highest distribution quartile). Associations were retested with single nucleotide polymorphism rs6742078, a genetic determinant of bilirubin.

Results

High bilirubin levels were significantly associated with higher FEV_1/FVC and FEF_{25-75} overall. Upon stratification, significant associations persisted in ever smokers, amounting to 1.1 percent (95%-confidence interval 0.1 to 2.2) increase in FEV_1/FVC, and 116.2 ml/sec (-15.9 to 248.4) in FEF_{25-75} per interquartile range of bilirubin exposure in smokers with high BMI. Associations were positive but non –significant in never smokers with high BMI. Similarly, rs6742078 genotype TT was associated with increased FEV_1/FVC and FEF_{25-75}.

Discussion

Our results suggest a possible protective role of bilirubin on lung tissue, which could be important for prevention and therapy.

INTRODUCTION

Bilirubin is an end product of heme degradation in the body which has raised considerable interest in research over the last decade.[1]

Increased serum bilirubin has repeatedly been associated with decreased risks for coronary artery disease, myocardial infarction, peripheral arterial disease, and stroke in retrospective and prospective clinical studies.[2, 3] The evidence for beneficial effects of elevated serum bilirubin was supported by animal and in vitro experiments showing anti-oxidative and anti-inflammatory properties[4].

Beneficial effects of elevated serum bilirubin have also been observed on respiratory health. Higher bilirubin concentrations were associated with lower incidence of lung cancer, chronic obstructive lung disease, and lung cancer mortality, as well as height-standardized lung function in previous population based studies .[5][6][7] These results suggest bilirubin might have protective effects in tissues exposed to the outer environment such as the lungs, possibly by counteracting subclinical inflammation.

Further, serum bilirubin levels were inversely associated with body mass index (BMI),[8] and were found to be lower in smokers than never smokers and decreased with higher smoking duration or intensity.[9]

The major genetic determinant of serum bilirubin is *UDP-glucuronosyltransferase 1A1 (UGT1A1).*[10] The enzyme degrades bilirubin by glucuronidation and allows its subsequent excretion into the bile. Polymorphisms in *UGT1A1* explain 10-30% of serum bilirubin variation.[1, 11] A functional repeat polymorphism in the promoter region underlies Gilbert syndrome which is prevalent in 5-10% of Caucasians: Compared to the wild-type allele consisting of six thymine-adenosine dinucleotides (TA) on each chromosome, persons homozygous for seven TA repeats have a 70% decreased UGT1A1 activity.[11] A genome wide study on bilirubin levels suggested that single nucleotide polymorphism (SNP) rs6742078 near UGT1A1 is in high linkage disequilibrium with the functional promoter repeat polymorphism, with its T-allele increasing bilirubin concentration.[10]

Eight studies attempted to corroborate causality for the observed associations between bilirubin and cardiovascular outcomes using Mendelian randomization, an instrumental variable analysis method, in which *UGT1A1* variants are analyzed to get unbiased estimates of bilirubin effects.[12-19] The most recent one on 67'068 healthy controls and 11'686 cases with ischemic heart disease observed no significant association with genetically elevated bilirubin, even after meta-analysis with the earlier studies.[19]

No comparable genetic studies have been conducted in the respiratory field, and the potential role of bilirubin in respiratory health has hardly been assessed. We thus aimed to study whether bilirubin serum levels were associated with lung function in the general population sample of the SAPALDIA cohort study. The level of lung function is an early pre-clinical marker of underlying pathologic lung processes such as inflammation and tissue remodeling. We a priori tested whether the relationship between bilirubin and lung function was altered by increased vulnerability arising from active tobacco smoking or subclinical inflammation related to excessive body weight. To assess causality for observed bilirubin associations, we studied the association of variant rs6742078 near *UGT1A1*with lung function in a subset of the population.

METHODS

Design and study population

The Swiss study on air pollution and health in adults (SAPALDIA) is a cohort study recruiting adults aged 18-60 years from eight Swiss communities. The SAPALDIA methods have been described in detail previously.[20] The present study is based on 4195 participants attending both examinations, with lung function testing, and with complete data on smoking exposure, and bilirubin measurement (**Figure 1**).

Written informed consent was obtained from all study participants, and the study was approved by the Swiss Academy of Medical Sciences as well as the respective regional ethics committees.

Health questionnaire

All study participants underwent an interview on smoking behavior, exposure to second hand smoke, occupational exposures, pre-existing respiratory, allergic and cardiovascular diseases and symptoms, as well as socio-economic factors.

Education comprised highest attained school degree until follow-up examination and was grouped into three categories: low (primary school only), medium (high school), and high educational level (university or college degree).

Never-smokers were defined as having smoked less than 20 packs of cigarettes or 360g of tobacco during lifetime. Former smokers had quit smoking at least one month before examination, and current smokers reported active smoking at the time of interview. In ever-smokers, packyears of smoking were calculated by dividing the number of cigarettes per day by 20, giving cigarette packs per day, which were multiplied by years of exposure.

Preexisting cardiopulmonary disease was defined as self-declaration of heart disease, lung emphysema, or chronic bronchitis, or use of health services for respiratory problems during the 12 months prior to follow-up examination. The detailed questions are given in the web-only file '**Supplemental methods**'.

Body mass index and spirometry

Participants wore no shoes or heavy clothes for the measurement of weight and height. Body mass index (BMI) was calculated by dividing weight (in kilograms) by the square of height (in meters).

Lung function testing was done using Sensormedics devices (Sensormedics model 2200, Yorba Linda, USA). Devices were calibrated daily, and comparability between devices was checked. Measurements were conducted according to the protocol of the European Community Respiratory Health Survey. Participants performed three to eight forced expiratory maneuvers while sitting in an upright position. Maneuvers had to comply with American Thoracic Society quality criteria,[21] and at least two acceptable values for the forced expiratory volume in the first second of exhalation (FEV_1), and forced vital capacity (FVC) were recorded. The ratio of FEV_1/FVC was derived from measurements of the same maneuver. Mean forced expiratory flow calculated between 25% and 75% of FVC (FEF_{25-75}) was recorded from the curve displaying the highest sum of FEV_1 and FVC.

Biomarkers

Whole blood samples were collected in 2002, and serum blood markers were measured using a Modular P Autoanalyser (Roche Diagnostics, Rotkreuz, Switzerland). Total bilirubin was determined by the diazo method.[22] Liver enzyme activities were determined by enzymatic UV-tests (including pyridoxalphosphate) for alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and by photometric test for γ-glutamyl transferase (GGT). Coefficients of variation ranged from 1 to 3%.

Bilirubin values above 17 µmol/L (upper normal value) with levels of ALT, AST, or GGT equaling or surpassing the double of the upper normal value (50, 52, and 66 U/L respectively) were set to missing due to possible liver disease.

Genotype data for rs6742078 near UGT1A1 was acquired in the genome-wide GABRIEL asthma study for 982 non-asthmatic individuals.[23, 24] Being non-asthmatic was defined as neither self-report nor doctors diagnosis of asthma using a standardized questionnaire.[20] Genotyping details are available in the web-only file '**Supplemental methods**'.

Definition of susceptible groups

Two susceptibility conditions were considered regarding lung function. The first comprised ever-smoking. The second included high body mass index (BMI) because of the associated subclinical inflammation which might interfere with protective effects of bilirubin. For different reasons, this condition was defined statistically by using the highest quartile of the BMI distribution (BMI≥28.36 kg/m^2) as threshold: first, waist circumference, more closely related to subclinical inflammation was not available. Second, categorizing on the highest quartile was reasonable in absence of a validated BMI threshold to capture inflammation, as the common overweight definition of BMI≥25 kg/m^2 was met by a large proportion of our older aged study population (reflected by a mean BMI of 25.7 kg/m^2). Obesity (BMI≥30 kg/m^2) was not chosen because of low sample size and its mechanical effects on lung function. Categorizing BMI also enabled subgroup definitions in combination with ever smoking.

We defined three susceptibility groups comprising never smokers with high BMI, ever-smokers without, and ever-smokers with high BMI. Never smokers without high BMI served as reference group.

Statistical analysis

The distribution of sex, age, educational level, smoking status, packyears smoked, height, weight, BMI, FEV_1, FVC, FEV_1/FVC, FEF_{25-75}, pre-existing cardiopulmonary disease, bilirubin, c-reactive protein levels, and genotypes of *UGT1A1* SNP rs6742078 was tabulated for the whole study population and susceptible groups. To account for differential distribution of sex- and age, adjusted bilirubin mean concentrations and standard errors were calculated for each group by predicting the values from a multiple linear regression model of log bilirubin on sex, age and group membership. Predictions were exponentiated to give units of µmol/L.

Bilirubin values were transformed using natural logarithm to achieve a more symmetric distribution. The cross-sectional association between log-transformed bilirubin values and FEV_1, FVC, FEV_1/FVC and FEF_{25-75} from year 2002 was assessed using multiple linear regression adjusting stepwise for sex, age, height and weight, then educational level and study area, and finally ever smoking status and packyears. To check for interactions, dummy variables coding susceptibility group membership and multiplicative terms with log bilirubin were included into the models. Analyses were then stratified by susceptibility groups. Never-smoking participants without high BMI served as reference.

As sensitivity analysis, participants with elevated bilirubin levels (but normal liver enzymes) were excluded to assess whether the observed associations persisted in the normal range of serum bilirubin. Analyses were also repeated after excluding participants taking asthma medication (defined as positive answer to the question "Are you currently taking any medicines including inhalers, aerosols or tablets for asthma?"). Finally, analysis was stratified by presence of cardiopulmonary disease to assess the role of pre-existing pathologies on the associations under study. Interaction was tested analogously with multiplicative interaction terms.

Mendelian randomization analysis is based on the idea that during meiosis, the alleles of a genetic locus are passed on to the next generation by chance. If the genetic locus highly influences the levels of a biomarker, this natural randomized experiment allows making inferences on the causality of biomarker effects.[25] Accordingly, the association between rs6742078 genotypes and serum bilirubin was tested using Wilcoxon ranksum tests. Associations between rs6742078 genotypes and lung function were tested in regression models with the same covariates as for serum bilirubin analysis.

Recessive models were analyzed (allele TT against GG/GT) to correspond to the functional impact of *UGT1A1* promoter $(TA)_7$ homozygosity.[3] In view of the small sample size (n=982) no stratified analysis was run.

All analyses were conducted using STATA version 10.1 (StataCorp, College Station, Texas, USA). Adjusted mean bilirubin concentrations and standard errors were predicted using the 'adjust' post-regression estimation command. Two-sided α-values of 0.05 and 0.1 were chosen as significance thresholds for main effects and interactions, respectively.

RESULTS

Characteristics of study population

Our study comprised 4195 subjects, of which 1503 never smokers without and 461 with high BMI, and 1713 ever smokers without and 518 with high BMI. Overall, there were 53.2% females, 22.1% current and 31.0% former smokers (**table 1**). Average age was 51.9 years, mean FEV_1, FVC and FEF_{25-75} were 3.2 L, 4.2 L, and 2.6 L/s respectively. Median tobacco smoke exposure in ever smokers was 17.0 packyears (interquartile range 6 to 35). Median serum bilirubin in all participants was 7 µmol/L (interquartile range 5 to 10 µmol/L). Across susceptibility groups, FEV_1/FVC and FEF_{25-75} decreased with high BMI and smoking exposure. Groups with high BMI were older and less educated, while more males were in the smoking groups. Bilirubin serum values were apparently comparable across the groups, but after adjusting for differences in sex and age distribution, concentrations decreased progressively from 7.84 to 6.77 µmol/L with increasing BMI and smoking exposure (**web-only file 'Table A1'**)..

Associations of bilirubin with lung function values

In the basic analysis models adjusting for sex, age, height and weight, FEV_1, FEV_1/FVC, and FEF_{25-75} were significantly and positively associated with serum bilirubin (**table 2**). One log unit increase in bilirubin, which corresponds to a 2.72 fold increase in serum concentration, was associated with increases of (beta estimate, (95%-confidence interval)): 36.4 (6.7 to 66.1) ml FEV_1, 0.9 (0.5 to 1.4) percent FEV_1/FVC, and 115.2 (58.5 to 172.0) ml/sec FEF_{25-75}. Effect estimates decreased after adjustment for tobacco smoke exposure, which also resulted in loss of statistical significance for the FEV_1-association. In contrast, FEV_1/FVC- and FEF_{25-75}-associations remained significant with estimated increases of 0.5 (0.1 to 1.0) percent and 66.9 (11.4 to 122.5) ml/sec per log unit bilirubin. This corresponds to increases of 0.3 (0.1 to 0.7) percent in FEV1/FVC and 46.4 (7.9 to 84.9) ml/sec in

FEF_{25-75} over the observed interquartile exposure range (5 to 10 μmol/L bilirubin, i.e. a doubling concentration).

Table 1 Characteristics of the whole study sample and analysis subgroups

VARIABLES		ALL		Never smokers w/o high BMI		NEVER SMOKERS with high BMI		EVER SMOKERS w/o high BMI		EVER SMOKERS with high BMI	
		n	estimates	n	estimates	n	estimates	n	estimates	n	estimates
FEMALE SEX	[*number/%*]	4195	**2230/**53.2	1503	**910/**60.5	461	**287/**62.3	1713	**841/**49.1	518	**192/**37.1
AGE	*in years* [*mean/sd*]	"	**51.9/**11.5	"	**50.2/**12.1	"	**55.2/**11.8	"	**51.2/**11.0	"	**56.1/**9.5
EDUCATION											
low	[*number/%*]	4195	**274/***6.5*	1503	**70/***4.7*	461	**63/***13.7*	1713	**86/***5.0*	518	**55/***10.6*
medium	[*number/%*]	"	**2771/***66.1*	"	**971/***64.6*	"	**322/***69.8*	"	**1143/***66.7*	"	**335/***64.7*
high	[*number/%*]	"	**1150/***27.4*	"	**462/***30.7*	"	**76/***16.5*	"	**484/***28.3*	"	**128/***24.7*
BODY MEASURES											
height	*in m* [*mean/sd*]	4195	**1.7/**0.1	1503	**1.7/**0.1	461	**1.7/**0.1	1713	**1.7/**0.1	518	**1.7/**0.1
weight	*in kg* [*mean/sd*]	"	**73.5/**14.4	"	**67.7/**10.6	"	**88.0/**12.8	"	**69.2/**11.0	"	**91.4/**12.2
BMI	*in kg/m^2* [*mean/sd*]	"	**25.7/**4.3	"	**23.8/**2.6	"	**31.9/**3.5	"	**24.0/**2.6	"	**31.7/**3.1
SMOKING											
current smoker	[*number/%*]	4195	**929/***22.1*	1503	**0/***0.0*	461	**0/***0.0*	1713	**754/***44.0*	518	**175/***33.8*
packyears*	[*median/25./75. perc*]	"	**17/**6.0/35.0					"	**15.6/**5.5/32.2	"	**22.4/**9.5/42.0
COMORBIDITY											
cardiopulmon.**	[*number/%*]	4195	**605**/14.4	1503	**173**/11.5	461	**83**/18.0	1713	**243**/14.2	518	**106**/20.5
LUNG FUNCTION											
FEV_1	*in L* [*mean/sd*]	4195	**3.2/**0.8	1503	**3.2/**0.8	461	**2.9/**0.9	1713	**3.2/**0.8	518	**3.0/**0.8
FVC	*in L* [*mean/sd*]	"	**4.2/**1.0	"	**4.2/**1.0	"	**3.9/**1.1	"	**4.4/**1.0	"	**4.2/**0.9
FEV_1/FVC	*in percent* [*mean/sd*]	"	**74.7/**7.5	"	**76.2/**6.9	"	**74.7/**7.0	"	**74.0/**7.6	"	**72.8/**8.1
FEF_{25-75}	in L/sec [*mean/sd*]	"	**2.6/**1.1	"	**2.8/**1.1	"	**2.5/**1.1	"	**2.6/**1.1	"	**2.4/**1.2
BIOMARKERS											
bilirubin	*in µmol/L* [*median/25./75. perc*]	4195	**7/**5.0/10	1503	**7/**6.0/10	461	**7/**5.0/9	1713	**7/**5.0/10	518	**7/**5.0/9
CRP	*in µmol/L* [*median/25./75. perc*]	4184	**1/**0.5/2.3	1498	**0.8/**0.4/1.6	"	**1.9/**1.0/4.4	1707	**0.9/**0.5/2	"	**2/**1.0/3.9
GENOTYPES											
rs6742078 GG	[*number/%*]	982	**402/***40.9*	343	**126/***36.7*	112	**46/***41.1*	402	**173/***43.0*	125	**57/***45.6*
GT	[*number/%*]	"	**453/***46.1*	"	**171/***49.9*	"	**50/***44.6*	"	**180/***44.8*	"	**52/***41.6*
TT	[*number/%*]	"	**127/***12.9*	"	**46/***13.4*	"	**16/***14.3*	"	**49/***12.2*	"	**16/***12.8*

**in ever smokers only **self-declared heart-disease, lung emphysema, chronic bronchitis, or health care use for breathing problems in the last 12 months*

Table 2 Association of logarithmized bilirubin with lung function parameters in the whole study population

Outcome	Model covariates	*beta estimate*	*95%-confidence interval*	*p-value*
FEV_1 (in ml)	sex, age, height, weight	**36.4**	*6.7 to 66.1*	**0.016**
	all previous & education, study area	**38.0**	*8.4 to 67.6*	**0.012**
	all previous & ever-smoking, total packyears	**13.8**	*-15.5 to 43.2*	0.356
FVC (in ml)	sex, age, height, weight	**-6.4**	*-41.4 to 28.7*	0.722
	all previous & education, study area	**-4.9**	*-39.6 to 29.7*	0.781
	all previous & ever-smoking, total packyears	**-14.5**	*-49.3 to 20.4*	0.416
FEV_1/FVC (in %)	sex, age, height, weight	**0.9**	*0.5 to 1.4*	<**0.001**
	all previous & education, study area	**1.0**	*0.5 to 1.4*	<**0.001**
	all previous & ever-smoking, total packyears	**0.5**	*0.1 to 1.0*	**0.012**
FEF_{25-75} (in ml/sec)	sex, age, height, weight	**115.2**	*58.5 to 172.0*	<**0.001**
	all previous & education, study area	**118.0**	*61.9 to 174.1*	<**0.001**
	all previous & ever-smoking, total packyears	**66.9**	*11.4 to 122.5*	**0.018**

Estimates are per natural log unit increase in bilirubin.
Positive values mean higher lung function, negative lower.

For FEV_1/FVC, the interaction between log bilirubin and being an ever smoker with or ever smoker without high BMI was statistically significant ($p_{interaction}$ ranging from 0.007 to 0.023, data not shown). Interactions were also significant on FEF_{25-75} ($p_{interaction}$ from 0.012 to 0.086), except after packyears adjustment ($p_{interaction}$ from 0.111 to 0.163). Stratifying the analysis by susceptibility groups confirmed the interactions (**table 3**): no significant associations were present in never-smokers without high BMI. In never smokers with high BMI, higher, although non-significant beta estimates were observed for FEV_1, FEV_1/FVC and FEF_{25-75}. Associations between log bilirubin and FEV_1, FEV_1/FVC and FEF_{25-75} were significant in ever smokers without high BMI. They did not withstand packyears adjustment for FEV_1 and FEV_1/FVC (21.7 (-23.6 to 67.0) ml and 0.6 (-0.1 to 1.2) percent increase per log unit bilirubin, respectively), but only FEF_{25-75} (88.7 (3.6 to 173.8) ml/sec). Largest beta estimates were observed in ever smokers with high BMI across all FEV_1/FVC and FEF_{25-75} models. After packyears adjustment, each log unit bilirubin was significantly associated with 1.6 (0.1 to 3.2) percent higher FEV_1/FVC and marginally associated with 167.6 (-23.0 to 358.3) ml/sec higher FEF_{25-75}. Over the observed interquartile range of bilirubin, this corresponds to increases of 1.1 (0.1 to 2.2) percent FEV_1/FVC and 116.2 (-15.9 to 248.4) ml/sec FEF_{25-75}.

Sensitivity analyses

Neither excluding participants with bilirubin levels above the normal range nor those with asthma medication affected association patterns substantially (**Figure 2**). Significant associations were observed in both, persons with and without pre-existing cardiopulmonary disease (web-only file '**Table A2**'). For FEV_1/FVC, estimates were significantly higher in the diseased group for education and smoking adjusted models ($p_{interaction}$ 0.086 and 0069, respectively; data not shown).

Table 3 Association of logarithmized bilirubin with lung function parameters stratified by ever smoking and high BMI

Outcome	Model	Never smokers without high BMI (n=1503)		Never smokers with high BMI (n=461)		Ever smokers without high BMI (n=1713)		Ever smokers with high BMI (n=518)	
		Beta (95%-CI)	*p-value*	*beta (95%-CI)*	*p-value*	*beta (95%-CI)*	*p-value*	*beta (95%-CI)*	*p-value*
FEV_1 (in ml)	model1	**10.3** *(-36.5 to 57.0)*	0.667	**14.9** *(-76.2 to 106.0)*	0.748	**53.4** *(7.6 to 99.2)*	0.022	**78.5** *(-22.6 to 179.6)*	0.128
	model2	**13.0** *(-33.8 to 59.7)*	0.586	**22.4** *(-68.1 to 112.8)*	0.628	**53.9** (*7.9 to 99.8)*	0.022	**76.2** *(-25.4 to 177.8)*	0.142
	model3	-		-		**21.7** (*-23.6 to 67.0)*	0.348	**43.0** (*-57.9 to 144.0)*	0.404
FVC (in ml)	model1	**9.2** (*-47.2 to 65.6)*	0.749	**-11.7** (*-118.6 to 95.2)*	0.830	**4.0** (*-50.4 to 58.5)*	0.885	**11.7** *(-97.5 to 120.9)*	0.834
	model2	**14.2** (*-41.8 to 70.3)*	0.619	**-8.6** (*-113.6 to 96.5)*	0.873	**5.7** (*-48.4 to 59.7)*	0.837	**-1.4** *(110.2 to 107.5)*	0.980
	model3	-		-		**-7.9** *(-62.3 to 46.6)*	0.777	**-26.8** *(-135.9 to 82.3)*	0.630
FEV_1/FVC (in%)	model1	**0.1** *(-0.6 to 0.7)*	0.877	**0.4** *(-0.9 to 1.8)*	0.514	**1.1** *(0.4 to 1.8)*	**0.001***	**1.9** *(0.3 to 3.4)*	**0.018***
	model2	**0.0** *(-0.6 to 0.7)*	0.928	**0.6** *(-0.7 to 1.9)*	0.395	**1.1** *(0.4 to 1.8)*	**0.002***	**2.0** *(0.5 to 3.6)*	**0.008***
	model3	-		-		**0.6** *(-0.1 to 1.2)*	0.093*	**1.6** *(0.1 to 3.2)*	**0.033***
FEF_{25-75} (in ml/sec)	model1	**12.8** *(-77.5 to 103.1)*	0.781	**45.5** *(-129.0 to 220.0)*	0.609	**154.3** *(66.8 to 241.7)*	**0.001***	**202.3** *(9.1 to 395.5)*	**0.040***
	model2	**12.7** *(-76.9 to 102.2)*	0.782	**66.6** *(-106.7 to 240.0)*	0.451	**151.1** *(64.6 to 237.6)*	**0.001***	**215.6** *(25.1 to 406.0)*	**0.027***
	model3	-		-		**88.7** *(3.6 to 173.8)*	**0.041**	**167.6** *(-23.0 to 358.3)*	0.085

Estimates are per natural log unit increase in bilirubin. Positive values mean higher lung function, negative lower.

* statistically significant difference compared to never smokers without high BMI ($p_{interaction}<0.1$)

model1: adjusting for sex, age, height, weight

model2: adjusting additionally for education and study area

model3: adjusting additionally for packyears smoked

95%-CI: 95% confidence interval

Associations of rs6742078 T-alleles with serum bilirubin and lung function values

Serum bilirubin values differed significantly according to the number of rs6742078 T-alleles (**web-only file 'TableA3'**). Median concentrations were 6 and 7 µmol/L for gentoypes GG and GT, versus 13 µmol/L for homozygous T-alleles ($p_{Wilcoxon}<0.001$).

Homozygous carriers of the rs6742078 T-allele had significantly higher levels of FEV_1/FVC and FEF_{25-75} in progressively adjusted linear regression models (**table 4**), resulting in 1.7 (0.4 to 3.0) percent higher FEV_1/FVC and 192.5 (30.8 to 354.2) ml/sec higher FEF_{25-75} than GT/GG carriers after packyears adjustment. No significant associations were observed for FEV_1 and FVC.

Table 4 Associations of rs6742078 genotypes with lung function

OUTCOME	MODEL	Estimates for recessive effects of rs6742078 (allele TT vs GG/GT)*	
		Beta (*95%-CI)*	**p-value**
FEV_1	model1	27.7 (*-65.9 to 121.2)*	0.562
	model2	40.6 (*-52.6 to 133.8)*	0.394
	model3	34.7 (*-57.2 to 126.7)*	0.459
FVC	model1	-58.4 (*-162.0 to 45.2)*	0.269
	model2	-42.8 (*-145.8 to 60.2)*	0.415
	model3	-44.3 (*-147.3 to 58.7)*	0.399
FEV_1/FVC	model1	1.7 (*0.4 to 3.1)*	**0.013**
	model2	1.8 (*0.4 to 3.2)*	**0.010**
	model3	1.7 (*0.4 to 3.0)*	**0.013**
FEF_{25-75}	model1	190.7 (*22.9 to 358.4)*	**0.026**
	model2	205.6 (*40.6 to 370.7)*	**0.015**
	model3	192.5 (*30.8 to 354.2)*	**0.020**

* rs6742078 was coded as recessive effect to correspond with functional effects of the *UGT1A1* promoter polymorphism: (TA)7/7 vs (TA)6/6 and (TA)6/7. Genetic data was available for n=982 participants.

model1: adjusting for sex, age, height, weight
model2: adjusting additionally for education and study area
model3: adjusting additionally for ever smoking and packyears smoked
95%-CI: 95%-confidence interval

DISCUSSION

In the population based SAPALDIA study, we found significant positive associations between serum levels of bilirubin and lung function parameters FEV_1/FVC and FEF_{25-75} after adjusting for the effects of sex, age, education, height and weight, as well as tobacco smoke exposure. When stratifying the study population according to states of increased susceptibility, associations persisted only in participants with high BMI and were increased and significant in ever smoking strata. Our results were robust to sensitivity analysis, and consistent associations were observed for genetically elevated bilirubin (due to rs6742078).

These findings are coherent with previous epidemiological evidence showing beneficial effects of elevated serum bilirubin on respiratory outcomes.[5-7] They suggest beneficial effects on lung function, but refine their scope to persons with subclinical inflammation and oxidative stress exposure (or cardiopulmonary disease). The significant associations with FEF_{25-75} and FEV1/FVC might point to an inverse relationship with small airway obstruction. Due to their large surface area, small airways are an important compartment of chronic respiratory disease development [26, 27] where subclinical changes often manifest first. Accordingly, strongest associations presented at this site in our general population sample. Smoking adjustment decreased association estimates considerably which was expected given its known inverse relationships with lung function and serum bilirubin, resulting in positive confounding of the bilirubin lung function association.

We can currently only speculate about the mechanisms by which serum bilirubin might influence lung function. Intracellular mechanisms are probably more important in the lung than the known effects in serum and blood vessels, as bilirubin penetrates cell walls at physiological pH-values.[28] Besides scavenging oxidants, bilirubin inhibits membrane-bound NADPH-oxidase, one of the major intracellular sources of reactive oxygen species.[29] Further, bilirubin infusions have been shown to downregulate inflammation in murine lung injury models.[30]

Because of the cross-sectional design, the observed associations cannot be interpreted clearly regarding temporal relationship of outcome and exposure: the findings could arise from a protective effect of pre-existing elevated serum bilirubin, preventing tissue damage by inflammatory and oxidative stress reactions, or bilirubin levels could represent a reactive marker of the individual oxidative stress burden and ensuing bilirubin consumption. Our results rather suggest a causal association for different reasons: First, the observed associations between serum bilirubin levels and lung function were consistently replicated in participants with genetically elevated serum bilirubin. Second, associations grew stronger across increasing states of subclinical inflammation and oxidative stress (i.e. in persons with high BMI, ever-smokers, or both), and even persisted in persons with cardiopulmonary disease. This would probably not be the case for a reactive biomarker. Finally, we observed similar but less strong associations in the normal range of serum bilirubin.

The study had few limitations besides its cross-sectional design. Genotyping data was only available in a subset of the population. Our data was potentially affected by measurement error, as lung function was only tested twice. But the resulting misclassification would rather be random and primarily affect study power. In contrast, our study had also several strengths. The population based design allowed investigating lung function as a pre-clinical marker of disease processes. The large study sample and detailed data on blood markers, body size, and lifestyle factors allowed stratifying the analysis to study associations across subclinical conditions of inflammation and oxidative stress. This offered new insights into possible health related effects of bilirubin. Our large study sample also allowed successful conduct of different sensitivity analyses. Finally, our study benefitted of a standardized spirometry protocol and adherence to strict quality control criteria.

In conclusion, we found significant positive associations of serum bilirubin levels with lung function in persons with increased inflammation and oxidative stress. From a public health perspective, it would be important to clarify whether the observed relationship is causal using prospective studies including genetic information. Bilirubin could bear large preventive and therapeutic potential, given the prevalence of smoking and high BMI in Western populations.

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Figure legends

Figure 1

BMI: body mass index in kg/m^2.

High BMI is defined as being in the highest quartile of the observed BMI distribution (BMI≥28.36 kg/m^2)

Figure 1 Selection of study participants

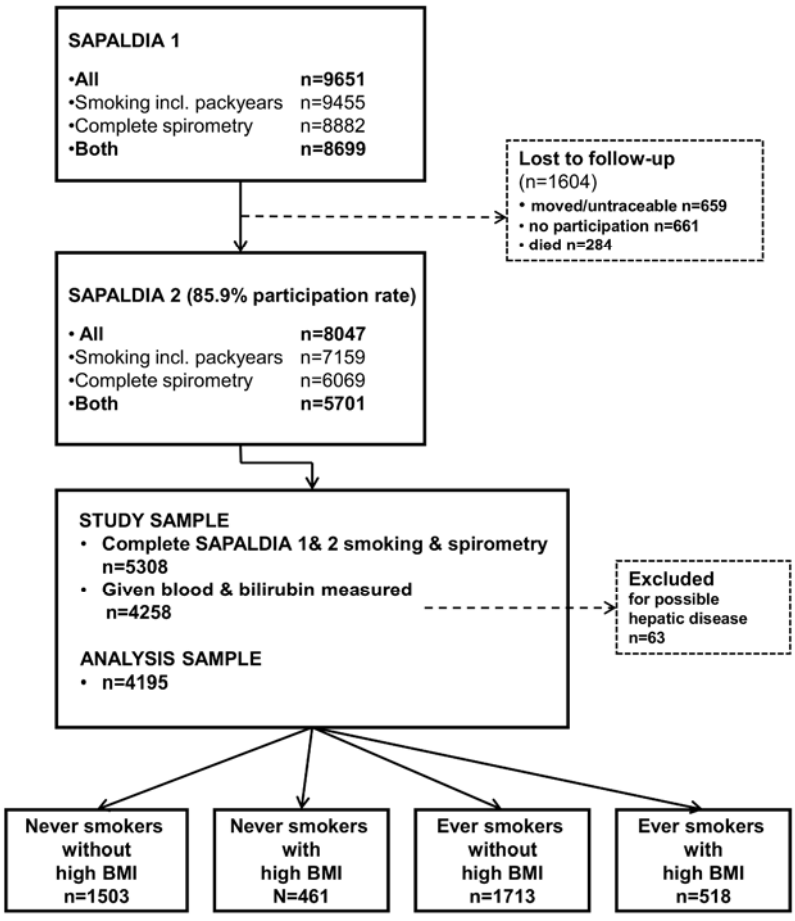
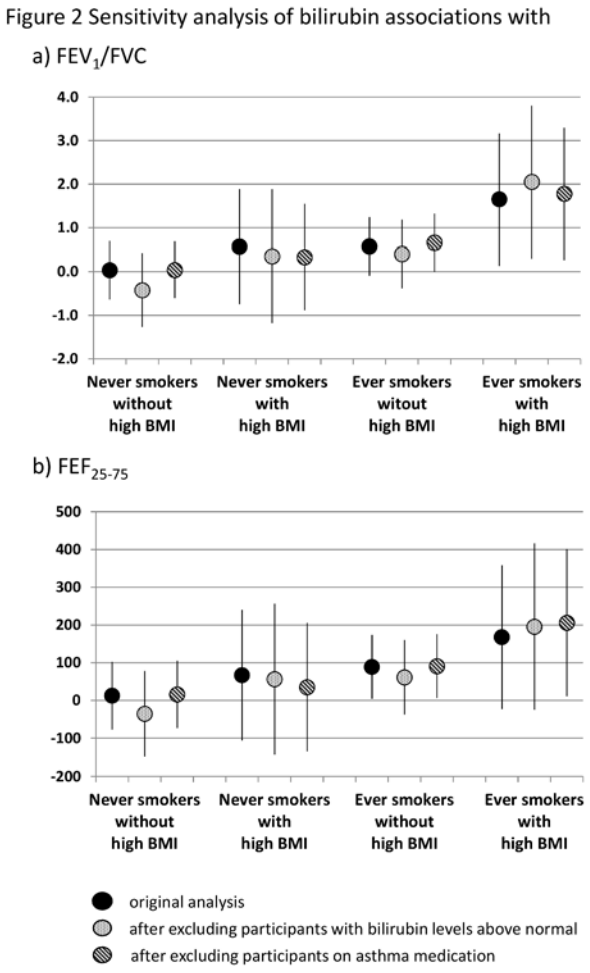


Figure 2

Estimates are indicated per log unit increase in bilirubin, and in units of percent for FEV1/FVC, and mL/sec for FEF_{25-75}.

Models adjust for sex, age, height, weight, education, study area, and in ever smoker strata additionally for packyears smoked.



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