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β_2-adrenergic receptor polymorphisms, asthma and COPD: two large population-based studies

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ABSTRACT: **The β_2-adrenergic receptor (ADRB2) is an important regulator of airway smooth muscle tone. We tested the hypothesis that three functional polymorphisms in the *ADRB2* gene (Thr164Ile, Gly16Arg and Gln27Glu) are associated with reduced lung function, asthma or chronic obstructive pulmonary disease (COPD).**

We first genotyped 8,971 individuals from the Copenhagen City Heart Study for all three polymorphisms. To validate our findings, we genotyped an additional 53,777 individuals from the Copenhagen General Population Study for the Thr164Ile polymorphism.

We identified 60,910 Thr164Ile noncarriers, 1,822 heterozygotes and 16 homozygotes. In the Copenhagen City Heart Study, the Thr164Ile genotype was associated with reduced forced expiratory volume in 1 s (FEV_1) % predicted (trend $p=0.01$) and FEV_1/forced vital capacity (FVC) ($p=0.001$): Thr164Ile heterozygotes had 3% and 2% reduced FEV_1 % pred and FEV_1/FVC, respectively, compared with noncarriers. The odds ratio for COPD in Thr164Ile heterozygotes was 1.46 (95% CI 1.05–2.02). In the Copenhagen General Population Study, the Thr164 genotype associated with reduced FEV_1 % pred ($p=0.04$) and FEV_1/FVC ($p<0.001$): Thr164Ile homozygotes and heterozygotes had 7% and 1% reduced FEV_1 % pred and 6% and 1% reduced FEV_1/FVC, respectively, compared with noncarriers. The odds ratios for COPD in Thr164Ile homozygotes and heterozygotes were 4.53 (95% CI 1.54–13.3) and 1.07 (95% CI 0.92–1.25), respectively.

Our results suggest that *ADRB2* Thr164Ile is associated with reduced lung function and increased risk of COPD in the general population.

KEYWORDS: **Asthma, β_2-adrenergic receptor, chronic obstructive pulmonary disease, genetics**

The β_2-adrenergic receptor (ADRB2) is a G protein-coupled transmembrane receptor located on airway smooth muscle cells [1]. Receptor activation causes smooth muscular relaxation in response to endogenous catecholamines, and this is important for the regulation of airway smooth muscle tone. Pharmacological targeting of this receptor is a widely used therapeutic approach for controlling bronchoconstriction associated with asthma and chronic obstructive pulmonary disease (COPD) [1].

There are three known polymorphisms in the coding region of the *ADRB2* gene that alter the function of the receptor [2, 3]. The rare variant, Thr164Ile, reduces the receptor–ligand binding affinity [1, 2], whereas the two common polymorphisms, Gly16Arg and Gln27Glu, determine the extent of receptor downregulation following agonist exposure [1, 3].

Several studies have investigated these polymorphisms and related haplotypes to assess their potential contribution to risk of asthma and COPD. The majority of studies have examined their relationship with risk of asthma, and have found positive associations with airway reactivity [4], nocturnal asthma [5] and asthma severity [6]. However, a large population-based study and meta-analyses have shown conflicting results [7–10]. For COPD, a single study found an elevated risk of disease for Arg16 homozygotes and for carriers of the Arg16/Gln27 haplotype [11], but these results have not been replicated by others [12, 13]. Larger sample sizes may be needed to detect small effect sizes of the *ADRB2*

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polymorphisms and to determine association with the rare Thr164Ile polymorphism.

We tested the hypothesis that genetic variation in the β_2-adrenergic receptor is associated with reduced lung function, and increased risk of asthma or COPD. For this purpose, we genotyped 8,971 individuals from the Copenhagen City Heart Study for the three most important functional polymorphisms in the *ADRB2* gene, Thr164Ile, Gly16Arg and Gln27Glu. Because previous studies found that genetic effects of *ADRB2* polymorphisms may be influenced by tobacco smoke [4, 14, 15], we also performed the statistical analyses stratified for tobacco smoking. To validate our findings, we genotyped an additional 53,777 individuals from the Copenhagen General Population Study for the Thr164Ile polymorphism. These two cohorts have been successfully used in previous genetic epidemiological studies, where positive associations between variants and disease have been found [16–18] and in others where possible associations have been excluded [19–21].

MATERIAL AND METHODS

We studied randomly selected white individuals of Danish descent from the Copenhagen City Heart Study and the Copenhagen General Population Study, two similar studies both recruited from the adult Danish general population. Individuals were selected from the national Danish Civil Registration System to give a population aged 20–80 yrs. Details of the selection procedure and study subjects have been given elsewhere (online supplementary material). The studies were approved by Herlev Hospital (Copenhagen, Denmark) and Danish ethical committees, and were conducted according to the Declaration of Helsinki. Written informed consent was obtained from all participants.

The Copenhagen City Heart Study

This prospective general population study was initiated in 1976 and up until now the participants have been invited to four examinations. At each examination, a questionnaire was completed concerning lifestyle factors and physical measurements were taken. At the third examination in 1991–1994, blood samples were drawn for DNA extraction: 9,259 samples were available for genotyping, and 9,246 individuals were genotyped for the Thr164Ile polymorphism, 9,245 for the Gly16Arg polymorphism and 9,243 for the Gln27Glu polymorphism. Of those, 163 individuals having an ethnic background other than Danish and 112 individuals lacking participant characteristics were excluded from the sample prior to statistical analysis.

The Copenhagen General Population Study

This study is a cross-sectional study of the Danish general population initiated in 2003 and still recruiting. All participants filled out a questionnaire, had physical measurements taken and blood was drawn for DNA isolation: 56,660 samples were available for genotyping and 56,599 individuals were genotyped for the Thr164Ile polymorphism. Of those, 2,822 individuals lacking participant characteristics were excluded from the sample prior to statistical analysis.

Genotyping

DNA was isolated from whole blood and stored at -80°C until time of genotyping. Genotypes were assigned continuously using smaller batches of data. Genotyping was performed at the laboratory in the Dept of Clinical Biochemistry at Herlev Hospital. A Nanogen NMW 1000 Nanochip Molecular Biology Workstation (Nanogen Inc., San Diego, CA, USA) was used to genotype the Thr164Ile (rs1800888), Gly16Arg (rs1042713) and Gln27Glu (rs1042714) polymorphisms in the *ADRB2* gene in the Copenhagen City Heart Study [22, 23]. In the Copenhagen General Population Study, we used a TaqMan-based assay (Applied Biosystems Inc., Foster City, CA, USA) to genotype participants for the Thr164Ile polymorphism (online supplementary material). Homozygotes for the rare allele were genotyped twice for verification. Because we performed reruns, call rates were >99.9% for all three polymorphisms.

Outcomes

Forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) were determined before administration of a bronchodilator using a dry-wedge spirometer (Vitalograph, Maids Moreton, UK) in the Copenhagen City Heart Study and an EasyOne Spirometer (ndd Medizintechnik, Zurich, Switzerland) in the Copenhagen General Population Study. Each spirometry was performed in triplicate, and the best results were used in the analyses. A total of 62,748 individuals had spirometry performed. Reference values were derived separately for each study using multiple regressions with age and height as covariates for all individuals, for males and females separately. COPD was defined as $FEV_1/FVC < 0.70$. Individuals taking asthma medication were excluded from the analyses concerning lung function and COPD. Asthma diagnoses were based on three questions, as follows. Asthma: ''do you have asthma?'' allergic asthma: ''does exposure to foodstuffs, medicine, flowers, animal hair or other things give you asthma?''; asthma medication: ''do you take medication for asthma/bronchitis daily or almost daily?'' Ever-smokers were defined as self-reported current smokers or ex-smokers.

Statistical analysis

Statistical analyses were performed using STATA/SE version 10.1 (StataCorp, College Station, TX, USA). A two-sided p-value $\leqslant 0.05$ was considered significant. We analysed the relationship of Thr164Ile, Gly16Arg and Gln27Glu genotypes with FEV_1 % predicted and FEV_1/FVC using Cuzick's test for trend and compared minor allele carriers with noncarriers using unpaired t-tests. We tested for possible interactions between the *ADRB2* genotype and ever smoking in predicting FEV_1 % pred and FVC % pred in ANCOVA models. Because repeated measurements of FEV_1 and FVC were available for subjects in the Copenhagen City Heart Study (30% of participants had four measurements, 39% three measurements, 21% two measurements and 10% one measurement), lung function was also analysed in a repeated-measures model using the mixed modelling option in SPSS version 17.0 (IBM, Lyngby, Denmark). The design of the Copenhagen City Heart Study specified repeated measurements of lung function. An unstructured covariance type for residuals was used. The unstructured type places no restrictions on the structure and may be preferable to other types. No random effects were specified. The Thr164Ile genotype and number of examinations of the Copenhagen City Heart Study were specified as fixed effects, and lung function was the repeated dependent variable. We analysed the relationship between *ADRB2* genotype and risk of asthma and COPD by logistic regression adjusted for age and sex. We performed haplotype analysis using the hapipf and qhapipf commands in STATA.

RESULTS

The Copenhagen City Heart Study

Characteristics of the 8,971 individuals tested for the Thr164Ile, Gly16Arg and Gln27Glu polymorphisms are shown in table 1. Distributions of sex, age, smoking status, pack-yrs, exposure to occupational dust or fumes and passive smoking were similar in the genotype groups. Genotype frequencies did not differ from those predicted by the Hardy–Weinberg equilibrium (Thr164Ile, p=0.46; Gly16Arg, p=0.06; Gln27Glu, p=0.18).

Lung function

Thr164Ile genotype was associated with reduced FEV_1 % pred (trend from noncarriers through heterozygotes to homozygotes, p=0.01) and FEV_1/FVC (p=0.001): Thr164Ile heterozygotes had 3% and 2% reduced FEV_1 % pred and FEV_1/FVC, respectively, compared with noncarriers (fig. 1). FEV_1 % pred and FEV_1/FVC did not differ by Gly16Arg genotype (p=0.80 and p=0.86, respectively) or Gln27Glu genotype (p=0.52 and p=0.11, respectively). To examine whether tobacco smoking added to reduce lung function, we stratified the analysis for smoking status. Among ever-smokers, Thr164Ile genotype was associated with reduced FEV_1 % pred (p=0.02) and FEV_1/FVC (p=0.001): Thr164Ile heterozygotes had 4% and 3% reduced FEV_1 % pred and FEV_1/FVC, respectively, compared with noncarriers (fig. 2). Among never-smokers, Thr164Ile genotype was associated with reduced FEV_1/FVC (p=0.01), but not with FEV_1 % pred (p=0.06): Thr164Ile heterozygotes had 2% reduced FEV_1/FVC compared with noncarriers and a trend towards reduced FEV_1 % pred (p=0.06). FEV_1 % pred and FEV_1/FVC did not differ by Gly16Arg and Gln27Glu after stratification for smoking status (data not shown). There was no interaction between Thr164Ile, Gly16Arg and Gln27Glu genotypes and ever smoking in predicting FEV_1 % pred or FEV_1/FVC.

Figure 3 shows the time course of FEV_1 % pred and FEV_1/FVC by Thr164Ile genotype based on all available lung function measurements from the four examinations. Thr164Ile heterozygotes had lower FEV_1 % pred and FEV_1/FVC in the age range from 20 to 80 yrs (repeated measures: p=0.007 and p=0.001, respectively). A single smoking Thr164Ile homozygote had declines of 32% in FEV_1 % pred and 21% in FEV_1/FVC over 25 yrs from the 1976–1978 examination to the 2001–2003 examination.

COPD

Thr164Ile heterozygotes had an odds ratio for COPD of 1.46 (95% CI 1.05–2.02) compared with noncarriers (fig. 4). The corresponding odds ratios for Gly16Arg heterozygotes and homozygotes were 0.96 (95% CI 0.84–1.10) and 0.90 (95% CI 0.75–1.10) (trend p=0.35), and for Gln27Glu heterozygotes and homozygotes 1.08 (95% CI 0.94–1.25) and 1.12 (95% CI 0.94–1.33) (trend p=0.15), respectively. Among ever-smokers, Thr164Ile heterozygotes had an odds ratio for COPD of 1.61 (95% CI 1.13–2.29). The corresponding odds ratios for Gly16Arg heterozygotes and homozygotes among ever-smokers were 0.96 (95% CI 0.83–1.10) and 0.88 (95% CI 0.72–1.08) (trend p=0.26), and for Gln27Glu heterozygotes and homozygotes were 1.13 (95% CI 0.97–1.32) and 1.14 (95% CI 0.94–1.38) (trend p=0.15), respectively. If asthmatics were included in the analyses, the results were similar to those presented (fig. S1).

TABLE 1 Characteristics of participants: the Copenhagen City Heart Study

Genotype	Subjects n	Females/males n	Age yrs	Ever-smokers %	Smoking exposure pack-yrs	Occupational exposure to dust or fumes %	Passive smoking history %
Thr164Ile							
Thr/Thr	8702	4842/3860	57.8±15.1	78.3	19.1±22.1	18.6	83.8
Thr/Ile	268	145/123	57.9±15.3	73.1	18.6±24.2	21.0	80.8
Ile/Ile	1	1/0	56.0	100.0	18.8	100.0	100.0
Gly16Arg							
Gly/Gly	3501	1979/1522	57.7±15.1	78.8	19.0±22.3	18.1	83.8
Gly/Arg	4138	2267/1871	58.1±15.0	77.7	19.3±22.5	19.6	83.6
Arg/Arg	1331	741/590	57.3±15.5	77.4	18.2±20.8	17.4	84.2
Gln27Glu							
Gln/Gln	2852	1599/1253	57.5±15.4	77.0	18.6±22.4	19.0	83.6
Gln/Glu	4355	2401/1954	58.0±14.9	78.3	19.3±21.8	19.3	84.0
Glu/Glu	1761	986/775	57.8±15.1	79.6	19.1±22.6	16.3	83.2

Data are presented as mean±SD, unless otherwise stated. All characteristics were recorded at the time when blood was drawn for genotyping. Occupational exposure to dust or fumes was defined as answering yes to the question "have you been exposed to occupational dust or fumes?" Passive smoking history was defined as answering yes to the question "are there smokers among the people in your household?" or "have either of your parents smoked?"

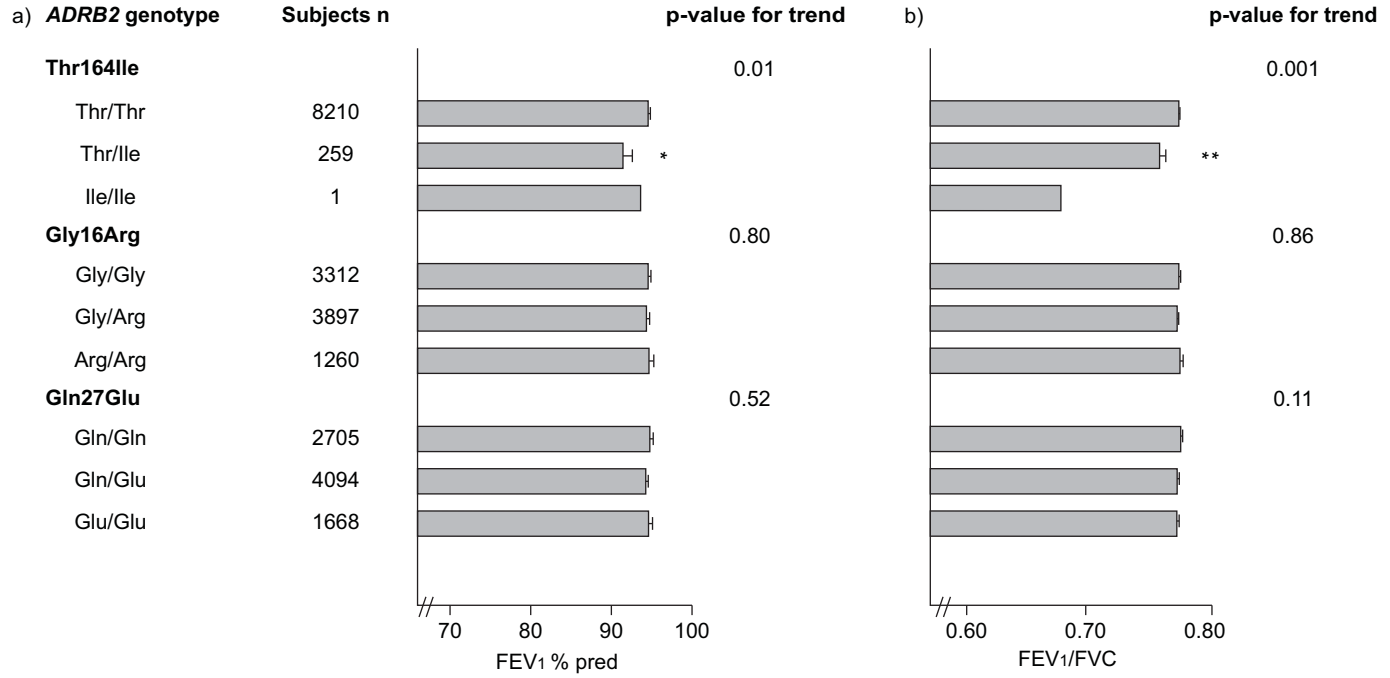

FIGURE 1. a) Forced expiratory volume in 1 s (FEV_1) % predicted (% pred) and b) FEV_1/forced vital capacity (FVC) according to *ADRB2* Thr164Ile, Gly16Arg and Gln27Glu genotype. Data are presented as mean±SE. Numbers are slightly less than those given in table 1 because not all individuals had spirometry performed. p-value for trend was determined using Cuzick's test for trend. *: $p<0.05$, t-test comparing Thr164Ile heterozygotes with noncarriers; **: $p<0.01$, t-test comparing Thr164Ile heterozygotes with noncarriers.

Asthma

Thr164Ile heterozygotes had an odds ratio for asthma of 0.72 (95% CI 0.40–1.29) compared with noncarriers (fig. 5). The corresponding odds ratios for allergic asthma and asthma medication were 0.71 (95% CI 0.43–1.16) and 0.57 (95% CI 0.29–1.12), respectively. Combining these, Thr164Ile heterozygotes had an odds ratio for any asthma of 0.76 (95% CI 0.52–1.11). Gly16Arg and Gln27Glu were not associated with risk of asthma, allergic asthma, asthma medication or any asthma (fig. 5). If COPD cases (1,548 individuals) were excluded from the analyses, results were similar to those presented (fig. S2).

Haplotype analysis

In order to describe the relationship between haplotypes and disease status, we performed haplotype analysis in the Copenhagen City Heart Study cohort. We found that the

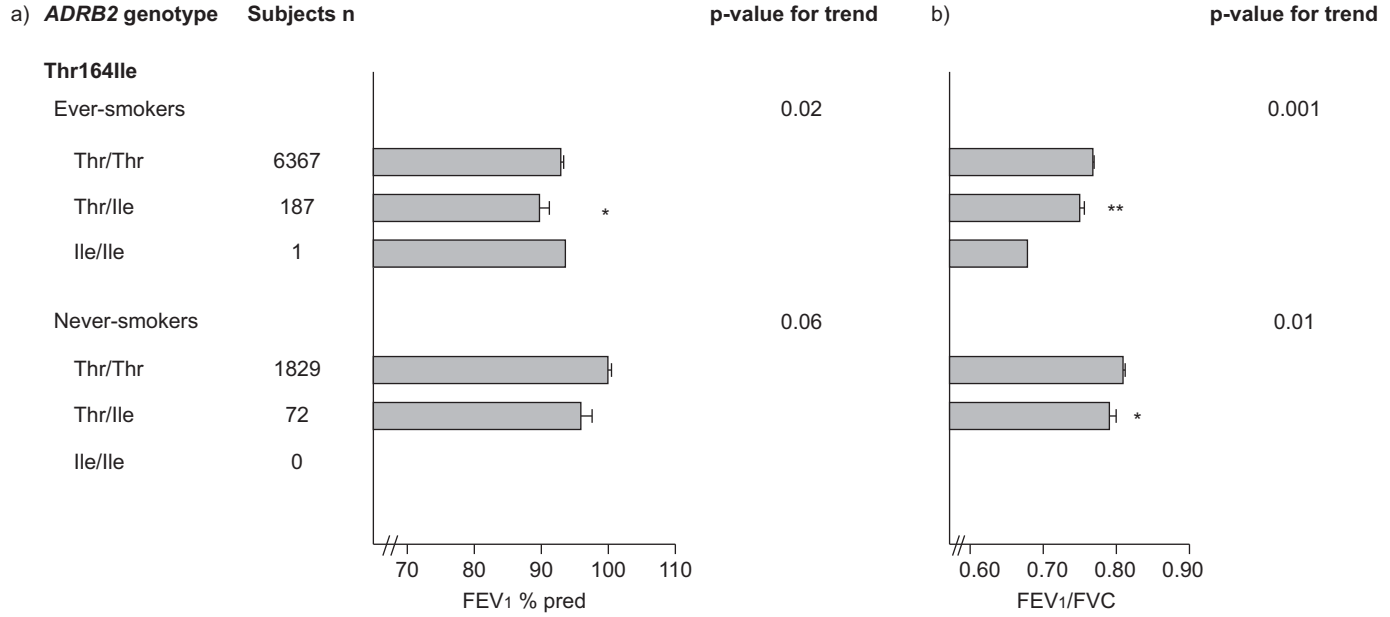

FIGURE 2. a) Forced expiratory volume in 1 s (FEV_1) % predicted (% pred) and b) FEV_1/forced vital capacity (FVC) in *ADRB2* Thr164Ile heterozygotes and homozygotes *versus* noncarriers stratified by smoking status. Data are presented as mean±SE. p-value for trend was determined using Cuzick's test for trend. *: $p<0.05$, t-test comparing Thr164Ile heterozygotes with noncarriers; **: $p<0.01$, t-test comparing Thr164Ile heterozygotes with noncarriers.

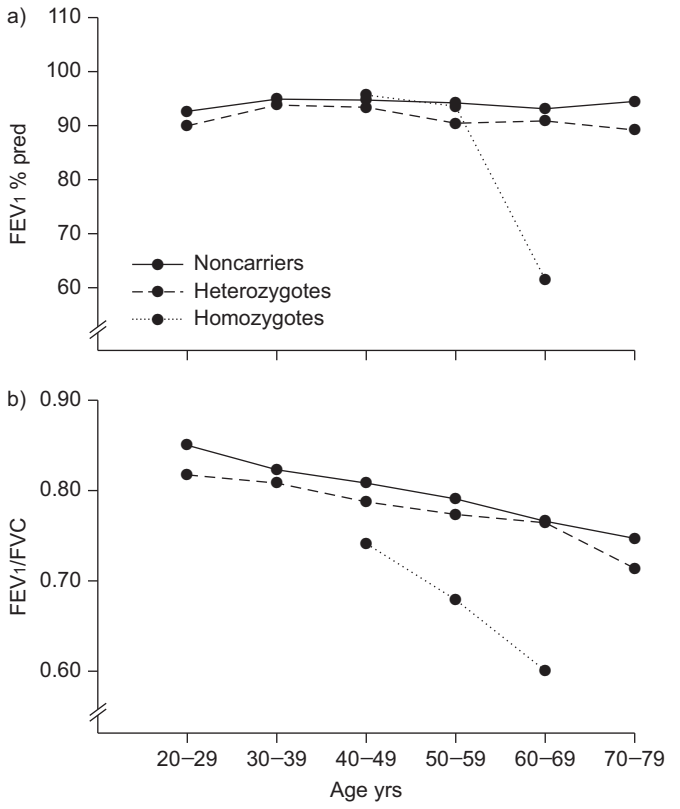

FIGURE 3. Course of a) forced expiratory volume in 1 s (FEV_1) % pred (% predicted) and b) FEV_1/forced vital capacity (FVC) by *ADRB2* Thr164Ile carrier status. Values are means based on 10-yr age groups up to 79 yrs. Thr164Ile noncarriers: n=24,850; heterozygotes: n=760; homozygotes: n=3. p-values were generated by general linear repeated-measures analysis comparing Thr164Ile heterozygotes *versus* noncarriers: a) p=0.007; b) p=0.001.

three-single nucleotide polymorphism haplotype was associated with FEV_1/FVC (p=0.007), but not with FEV_1 % pred (p=0.14), COPD (p=0.52), self-reported asthma (p=0.99), allergic asthma (p=0.99) or asthma medication (p=0.99).

The Copenhagen General Population Study

We also genotyped the Copenhagen General Population for the Thr164Ile polymorphism, in order to validate our findings. Characteristics of the 53,777 individuals tested for Thr164Ile are shown in table 2. Of those, 3,562 individuals taking asthma medication were excluded from the analyses on lung function and COPD, leaving 50,215 individuals. The analysis of self-reported asthma, allergic asthma and asthma medication included 53,777 individuals. Distributions of sex, age, smoking status, pack-yrs, exposure to occupational dust or fumes and passive smoking were similar between genotype groups. Thr164Ile genotype frequencies did not differ from those predicted by the Hardy–Weinberg equilibrium (p=0.51).

Thr164Ile genotype was associated with reduced FEV_1 % pred (trend p=0.04) and FEV_1/FVC (p<0.001): Thr164Ile homozygotes and heterozygotes had 7% and 1% reduced FEV_1 % pred and 6% and 1% reduced FEV_1/FVC, respectively, compared with noncarriers (fig. 6). When stratifying the analysis for smoking status, Thr164Ile was associated with reduced FEV_1/FVC in never-smokers (p=0.007) and ever-smokers (p=0.009), and with reduced FEV_1 % pred in never-smokers (p=0.04), but not in ever-smokers (p=0.21). There was no interaction between Thr164Ile genotype and ever-smoking in predicting FEV_1 % pred and FEV_1/FVC.

Thr164Ile homozygotes and heterozygotes had odds ratios for COPD of 4.53 (95% CI 1.54–13.3) and 1.07 (95% CI 0.92–1.25), respectively, compared with noncarriers. One out of 15 Thr164Ile homozygotes had allergic asthma, resulting in odds ratios for allergic asthma and any asthma of 1.19 (95% CI 0.16–9.09) and 0.67 (95% CI 0.09–5.12), respectively. Thr164Ile heterozygotes had odds ratios of 1.04 (95% CI 0.85–1.27) for asthma, 1.06 (95% CI 0.87–1.27) for allergic asthma, 1.03 (95% CI 0.84–1.25) for asthma medication and 1.05 (95% CI 0.89–1.23) for any asthma.

DISCUSSION

Asthma and COPD are common respiratory diseases caused by interaction of environmental risk factors with genetic background [17, 24, 25]. While several environmental risk factors

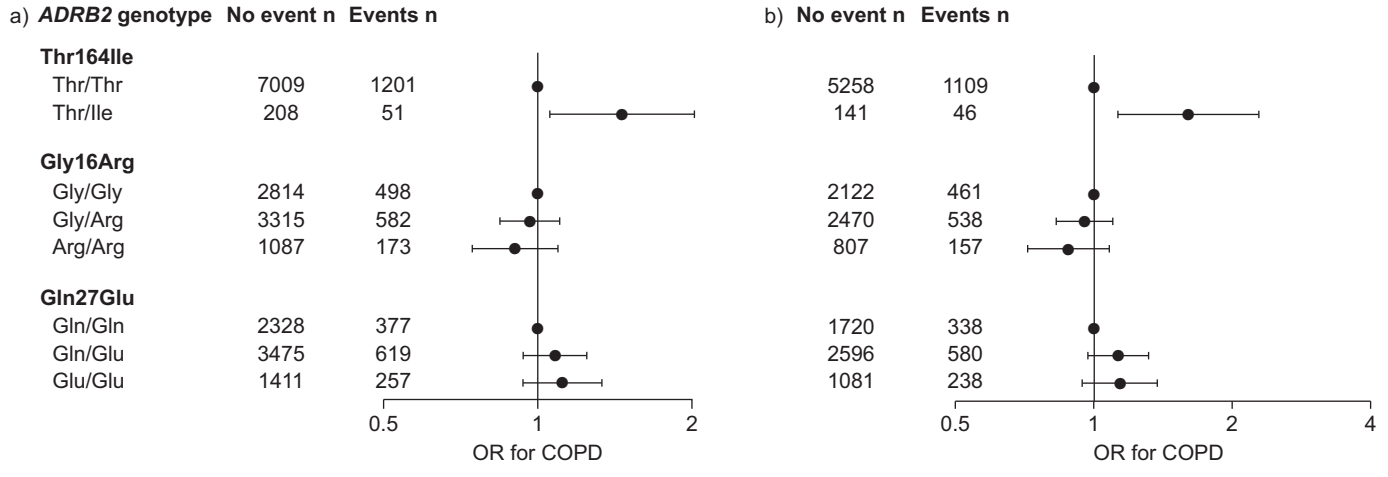

FIGURE 4. Risk of chronic obstructive pulmonary disease (COPD) in a) all subjects and b) ever-smokers only according to *ADRB2* Thr164Ile, Gly16Arg and Gln27Glu genotype. Data are presented as odds ratio (OR) and 95% confidence intervals (whiskers). The adjusted logistic regression model allowed for age and sex. COPD was defined as forced expiratory volume in 1 s/forced vital capacity <0.7 excluding individuals taking asthma medication.

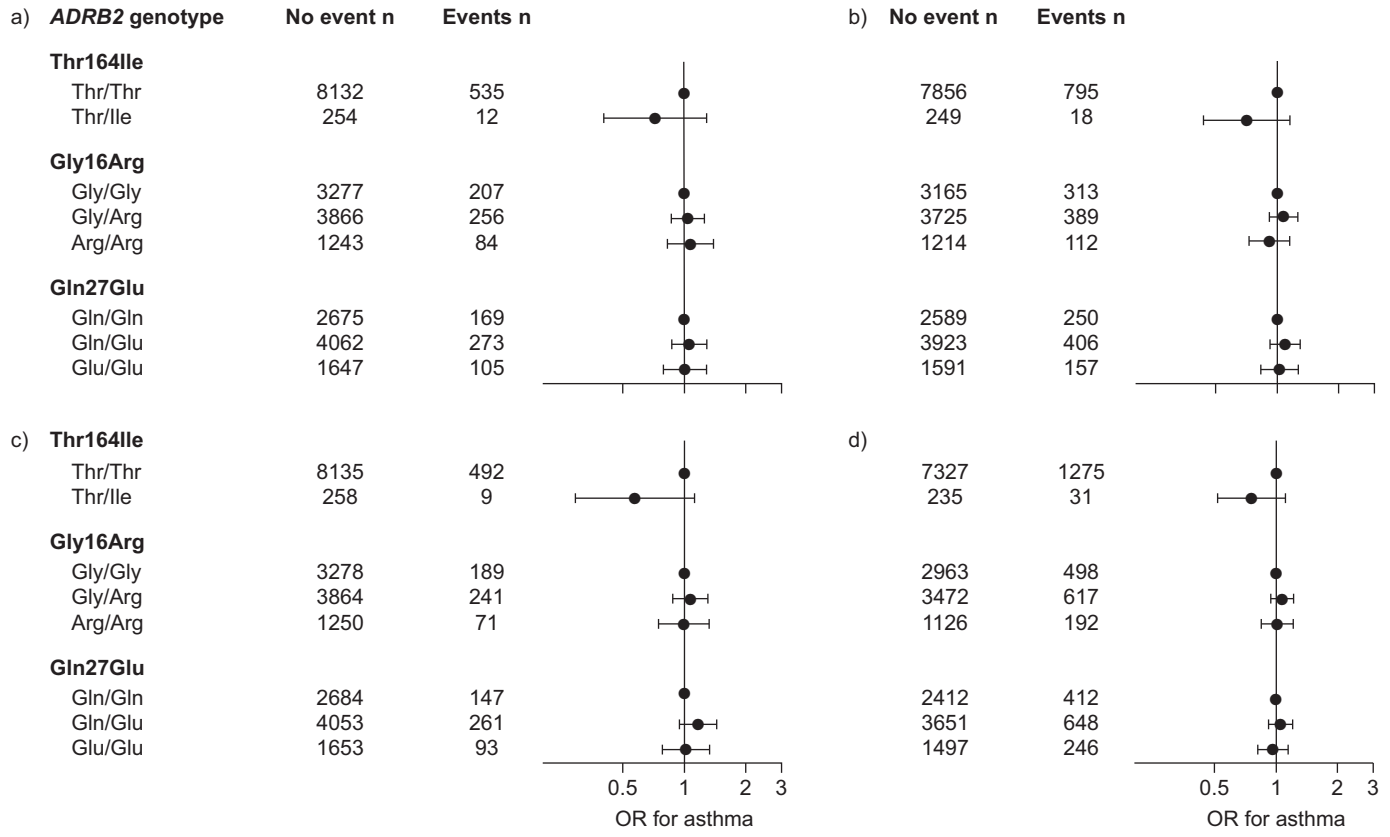

FIGURE 5. Risk of a) asthma, b) allergic asthma, c) asthma medication or d) any asthma according to *ADRB2* Thr164Ile, Gly16Arg and Gln27Glu genotype. Data are presented as odds ratios (OR) and 95% confidence intervals (whiskers). The adjusted logistic regression model allowed for age and sex. Asthma was defined as answering yes to the question ''do you have asthma?'' Allergic asthma was defined as answering yes to the question ''does exposure to foodstuffs, medicine, flowers, animal hair or other things give you asthma?'' Asthma medication was defined as answering yes to the question ''do you take medication for asthma/bronchitis daily or almost daily?'' Any asthma was defined as answering yes to at least one of these three questions.

have been identified, the genetic risk factors are less well understood. One possible risk gene for asthma and COPD is *ADRB2*, which encodes the β_2-adrenergic receptor. This receptor is expressed by airway smooth muscle cells and is an important pharmacological target in the management of asthma and COPD. Previous results on *ADRB2* variants in asthma and COPD have shown conflicting or negative results [1, 14]. Our study contributes results from the hitherto largest population-based investigation of *ADRB2* in asthma and COPD. We screened 8,971 people in the Copenhagen City Heart Study for Thr164Ile, Gly16Arg and Gln27Glu, the three polymorphisms with the highest reported effect on β_2-adrenergic receptor function. We found that Thr164Ile heterozygotes had reduced FEV_1 % pred and FEV_1/FVC and a greater risk of COPD compared with noncarriers. Using data from the Copenhagen General Population Study, we confirmed the associations of Thr164Ile with reduced FEV_1 % pred and FEV_1/FVC and with increased risk of COPD. Thr164Ile homozygotes and heterozygotes had 6% and 1% reduced FEV_1/FVC, and Thr164Ile homozygotes had also an elevated odds ratio for COPD of 4.5 (95% CI 1.5–13). None of the Thr164Ile, Gly16Arg and Gln27Glu polymorphisms were associated with asthma, allergic asthma or asthma medication in any of the two cohorts. These results suggest that *ADRB2* Thr164Ile is associated with reduced lung function and increased risk of COPD in the general population. The results also suggest that the three functional Gly16Arg, Gln27Glu and Thr164Ile polymorphisms are not major risk factors of asthma.

The lung function and COPD associations found for Thr164Ile, but not for Gly16Arg and Gln27Glu, could be due to Thr164Ile having the most profound functional consequences for the ADRB2 receptor. The substitution of isoleucine for threonine at position 164 lies next to a serine with predicted involvement in adrenergic ligand binding [1]. In concordance with this, studies using recombinant cells have found that the Thr164Ile allele has four times less ligand affinity and a 50% reduction in agonist-stimulated adenyl cyclase activity [2]. Similarly, Thr164Ile heterozygotes had a five-fold reduction in sensitivity to β_2-receptor agonist-mediated vasodilatation, and they had increased vasoconstrictor sensitivity [26]. These profound effects of Thr164Ile on smooth muscle constriction could potentially influence lung function and make the observed associations in our study biologically plausible.

Few previous studies have investigated the *ADRB2* polymorphisms in COPD in a Caucasian population and none of them have included the rare Thr164Ile polymorphism. MATHESON *et al.* [11] found an increased risk of COPD for Arg16 homozygotes and for the Arg16/Gln27 haplotype in an Australian Caucasian population (n=1,090). Contrasting with these findings, a case–control

TABLE 2 Characteristics of participants: the Copenhagen General Population Study

Genotype	Subjects n	Females/males n	Age yrs	Ever-smokers %	Smoking history pack-yrs	Occupational exposure to dust or fumes %	Passive smoking history %
Thr164Ile							
Thr/Thr	52208	29214/22994	59.8±13.5	61.3	8.6±15.4	10.9	23.2
Thr/Ile	1554	857/697	60.0±13.2	59.3	5.4±8.5	11.7	23.7
Ile/Ile	15	8/7	64.6±11.6	53.3	9.5±20.4	33.3	20.0

Data are presented as mean±SD, unless otherwise stated. All characteristics were recorded at the time when blood was drawn for genotyping. Occupational exposure to dust or fumes was defined as answering yes to the question ''have you been exposed to occupational dust or fumes?'' Passive smoking history was defined as answering yes to the question ''have you been exposed to passive smoking?''

study of 190 German COPD patients and 172 healthy controls found that the Gly16 allele was more common among cases than controls [13]. Other studies have also observed an association of Gly16 with COPD-related phenotypes [27, 28], and associations have also been observed between other *ADRB2* variants and reduced lung function and COPD [27, 29]. The present study could not replicate any of the previous positive associations found for Gly16Arg and Gln27Glu in COPD; however, in line with previous reports of a relationship between *ADRB2* and COPD, we did find associations of reduced lung function and COPD risk with the rare, and probably more severe, Thr164Ile genotype.

The role of the *ADRB2* gene in asthma has been controversial, with inconsistent findings. This could be due to differences in inclusion criteria and study end-points, numbers of study participants and/or ethnic background. Our study, using various definitions of asthma, found no increased risk of disease by the *ADRB2* genotype. In line with this, HALL *et al*. [10] conducted a similarly large study (n=8,018) using a British birth cohort and found that none of the three polymorphisms were important determinants of asthma incidence or prevalence in the British population. Our study could not examine association of *ADRB2* with specific asthma phenotypes, such as airway reactivity, nocturnal asthma and asthma severity, and we cannot totally exclude association of *ADRB2* with these end-points or with childhood asthma. A few previous studies have suggested an association of *ADRB2* with reduced lung function and wheezing among children [4, 15, 30]. In accordance with this, our results in figure 3 support that lung function in Thr164Ile carriers may be reduced in the age range 20–80 yrs, and perhaps earlier. Future studies would be required to conclusively determine whether *ADRB2* polymorphisms are related to reduced lung function in childhood.

The associations of Thr164Ile with reduced lung function and COPD were attenuated in the Copenhagen General Population Study as compared with the Copenhagen City Heart Study. The

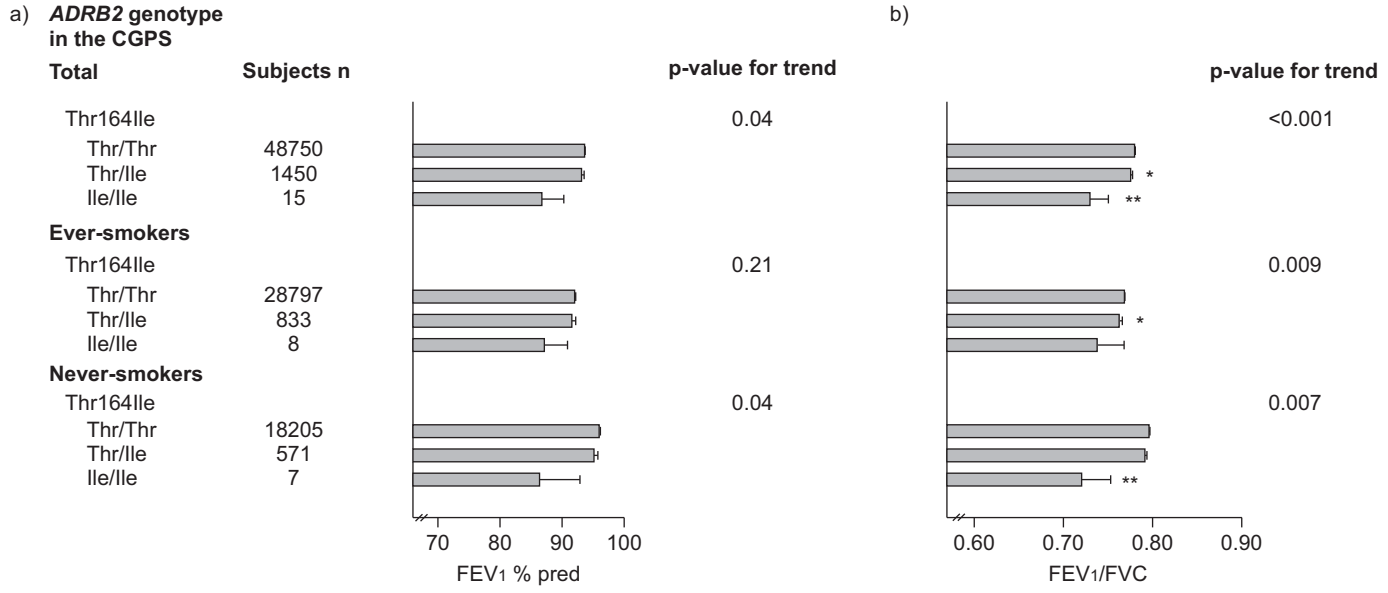

FIGURE 6. a) Forced expiratory volume in 1 s (FEV_1) % predicted (% pred) and b) FEV_1/forced vital capacity (FVC) in *ADRB2* Thr164Ile heterozygotes and homozygotes *versus* noncarriers in the Copenhagen General Population Study, overall or stratified by smoking status. Data are presented as mean±SE. Numbers are slightly less than those given in table 2 because not all individuals had spirometry performed. p-value for trend was determined using Cuzick's test for trend. *: $p<0.05$, t-test comparing Thr164Ile heterozygotes with noncarriers; **: $p<0.01$, t-test comparing Thr164Ile homozygotes with noncarriers.

Copenhagen General Population Study is currently recruiting subjects with lower daily tobacco consumption and better lung health status, which may partly explain the less pronounced effect on lung function and COPD in this study. However, although our two studies had limitations and potential biases based on their different study designs, the results were congruent. Replication is the gold standard in genetic epidemiological studies, and the associations of Thr164Ile with reduced lung function and increased risk of COPD in the Copenhagen City Heart Study were confirmed in an independent sample from the Danish population, the Copenhagen General Population Study.

In our study, some degree of misclassification of asthma was possible, since participants classified themselves. However, the prevalence of asthma was in accordance with the reference figures for Denmark and using alternative definitions of asthma, such as allergic asthma and asthma medication, showed similar results. All participants in this study were white and of Danish descent, which does not reflect today's ethnic pattern in the general population. We removed the few non-Danish participants from the Copenhagen City Heart Study prior to analysis. It is, therefore, unlikely that the association found could be due to systematic differences in allele frequencies in our cohorts caused by population stratification; however, our results may apply to Caucasians only. Our cohorts have previously been used in numerous studies focusing on genetic predictors of lung function and COPD and, theoretically, some of the associations found could be due to multiple comparisons. However, in our study, we tested a biologically plausible association in a well-known candidate gene, making the found association less likely to be due to chance. Bias caused by investigator knowledge of disease or risk factor status seems unlikely because our sample was selected from the general population and because genotyping of our sample was performed without investigator knowledge of disease status or lung function test results.

We have studied three of the polymorphisms (Thr164Ile, Gly16Arg and Gln27Glu) with the highest reported effect on β_2-adrenergic receptor function. By screening two large population-based studies for these polymorphisms, we found that the Thr164Ile variant is associated with reduced lung function and increased risk of COPD. Our results also suggest that the Thr164Ile, Gly16Arg and Gln27Glu polymorphisms are not related to risk of asthma.

STATEMENT OF INTEREST

None declared.

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