



Research Paper

Responsiveness to Ipratropium Bromide in Male and Female Patients with Mild to Moderate Chronic Obstructive Pulmonary Disease



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ARTICLE INFO

Article history:

Received 8 February 2017

Received in revised form 30 March 2017

Accepted 11 April 2017

Available online 12 April 2017

Keywords:

Sex

FEV₁

Ipratropium

COPD

Gene expression

Lung

ABSTRACT

Introduction: Although the prevalence of chronic obstructive pulmonary disease (COPD) is similar between men and women, current evidence used to support bronchodilator therapy has been generated in therapeutic trials that have predominately enrolled male patients. Here, we determined whether there is any significant sex-related differences in FEV₁ responses to ipratropium bromide.

Methods: Data from the Lung Health Study (n = 5887; 37% females) were used to determine changes in FEV₁ with ipratropium or placebo in male and female subjects with mild to moderate COPD over 5 years. Lung Expression Quantitative Trait Loci (eQTL) dataset was used to determine whether there were any sex-related differences in gene expression for muscarinic (M2 and M3) receptors in lungs of male and female patients.

Results: After 4 months, ipratropium therapy increased FEV₁ by 6.0% in female and 2.9% in male subjects from baseline values (p = 2.42 × 10⁻¹⁶). This effect was modified by body mass index (BMI) such that the biggest improvements in FEV₁ with ipratropium were observed in thin female subjects (p for BMI * sex interaction = 0.044). The sex-related changes in FEV₁ related to ipratropium persisted for 2 years (p = 0.0134). Female compared with male lungs had greater gene expression for M3 relative to M2 receptors (p = 6.86 × 10⁻⁸).

Conclusion: Ipratropium induces a larger bronchodilator response in female than in male patients and the benefits are particularly notable in non-obese females. Female lungs have greater gene expression for the M3 muscarinic receptor relative to M2 receptors than male lungs. Female patients are thus more likely to benefit from ipratropium than male COPD patients.

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1. Introduction

Traditionally, owing to greater cigarette smoking in men, chronic obstructive pulmonary disease (COPD) has been considered a “male disease”. However, with the marked rise in the smoking rates in women since the 1960's, there has been a sharp increase in the burden of

COPD among women throughout much of the Western world. Today, in the United States (US), there are 7 million more women than men with COPD and 10,000 more women than men die from COPD each year. Currently, the mortality rate is nearly 10 fold higher in women than in men (3.7%/year in females and 0.4%/year in males) (Ma et al., 2015). Despite this, ironically, the current management strategies for COPD (in both men and women) are largely based on therapeutic clinical trials that have recruited mostly male patients. In most therapeutic trials (even contemporary ones), female patients make up only 20%–25% of the total cohort (Calverley et al., 2007; Magnusson et al., 2014).

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There are some compelling biological reasons why there may be significant sex-related differences in the therapeutic responsiveness of inhaled drugs in COPD. Firstly, there are emerging data that indicate for the same severity of COPD, as measured by lung function, female patients have less emphysema and more small airways disease (Dransfield et al., 2007; Cazzola et al., 2011). Secondly, female patients with COPD demonstrate greater levels of bronchial reactivity to non-specific stimuli such as methacholine compared with male patients for the same degree of airflow limitation (Tashkin et al., 1992). Thirdly, women may have heightened xenobiotic metabolism of chemicals including those related to cigarette smoke and medications, as well different gene or protein expression (compared to men) of known drug targets and transporters which may modify the effectiveness of therapeutic drugs (Benowitz et al., 2006). Despite these considerations, it is not known whether there is any significant sexual dimorphism in the way in which men and women respond to commonly inhaled drugs in COPD (Statista, n.d.; FirstWord Pharma, 2013). The principal aim of the present study was to determine whether there are any sex-related differences in bronchodilation related to the use of inhaled ipratropium, one of the most commonly prescribed muscarinic receptor antagonist in the world, in patients with COPD (Statista, n.d.; FirstWord Pharma, 2013).

2. Methods

2.1. Data Source

To address the primary aim of the current study, we used data from the Lung Health Study (LHS). The details of LHS have been reported previously (Anthonisen et al., 1994). Briefly, LHS was originally designed to determine the effects of ipratropium and smoking cessation programs on the rate of decline in lung function over 5 years. At the time of recruitment all subjects were active smokers between the ages of 35 and 60 years (with a mean age of 48 years) who had smoked at least 10 cigarettes a day within the 30 days prior to initial screening and who demonstrated mild to moderate COPD on spirometry defined by forced expiratory volume in 1 s (FEV_1) of 55% to 90% of predicted, in the presence of FEV_1 /forced vital capacity (FVC) ratio of <0.70 after bronchodilation (mean FEV_1 of the cohort was 75% predicted and the mean FEV_1 /FVC was 63% post-bronchodilator).

After enrollment, these patients were randomly assigned to one of 3 groups: 1) usual care (UC), who received no intervention, $n = 1964$; 2) an intense anti-smoking (special) intervention and ipratropium bromide (Atrovent®, Boehringer Ingelheim Pharmaceuticals) $n = 1961$ (SIA); or 3) an intense anti-smoking (special) intervention and an inhaled placebo, $n = 1962$ (SIP). Subjects were excluded if they had serious co-morbidities that could have interfered with follow-up or impacted lung function (e.g. overt heart failure, ischemic heart disease, or malignancy other than skin cancer). Ten centres participated in the original study and together they recruited 5887 patients (of whom 37% were females). Those who were in the SIP or SIA groups received a program that consisted of: 1) a strong recommendation by attending physician for smoking cessation in an one-to-one encounter; 2) a group program led by a health educator that met 12 times over 10 weeks, which taught behaviour modification techniques; and 3) a nicotine replacement therapy with nicotine gum (Nicorette Gum, Marion Merrell Dow Inc), which was provided at no cost to patients. Those who successfully quit smoking were enrolled in a maintenance program to prevent relapses.

2.2. Spirometry and Follow-up

At the baseline visit, a detailed history of respiratory symptoms and smoking status was obtained. These included years of smoking, number of pack-years of smoking, current number of cigarettes smoked per day and the age of initiation. Additionally, all subjects underwent

spirometry, which was performed according to the standards of the American Thoracic Society. Bronchodilator response was measured after 2 inhalations of isoproterenol (200 µg total dose).

2.3. Interventions

Patients who were assigned to ipratropium or inhaled placebo were instructed on the use of metered dose inhalers by a physician and were asked to use 2 puffs (36 µg) of the inhaler 3 times a day. All subjects were brought back 4 months following randomization to encourage inhaler compliance and to determine smoking status. Smoking status was ascertained by detailed self-report that included the number of cigarettes smoked per day and exposure to any other forms of nicotine and objectively validated by measuring exhaled carbon monoxide (at every visit) and salivary cotinine (at annual visits) using well-validated immunoassays. The cutoff of exhaled carbon monoxide was 10 parts per million (ppm) to denote active smoking and that for salivary cotinine was 20 ng/ml. All subjects who exceeded any of these thresholds were classified as smokers. These objective measures of smoking status were blinded to patients. Non-smoking was defined at each annual visit by abstinence from all smoked tobacco products, validated by salivary cotinine and exhaled carbon monoxide. Sustained quitters were defined as non-smoking at all annual visits from year 1 through year 5. Continuous smokers were defined as smokers at all annual visits and intermittent quitters were defined as those who were nonsmokers in at least one annual follow-up visit (Anthonisen et al., 1994). At annual visits, in addition to the above, spirometry was performed before and after 2 puffs of isoproterenol. Compliance with inhalers was assessed by self-report and by weighing the canister. Follow-up was successful in 90% of patients over these 5 years.

2.4. Gene expression for M2 and M3 Muscarinic Receptor Subtypes in Lungs

One important factor that may determine responsiveness to inhaled therapeutic drugs is the receptor expression for the ligand in lung tissue. As the LHS did not obtain lung tissue, we used data from the Lung Expression Quantitative Trait Loci (eQTL) study (Hao et al., 2012) to determine whether there was a sex-related difference in the gene expression for M3 (CHRM3) and M2 (CHRM2) muscarinic receptor in lungs of individuals, who underwent lung resection surgery. The M3 receptor is the primary muscarinic receptor subtype that induces bronchial smooth muscle contraction; whereas presynaptic M2's are autoreceptors that prevent the release of acetylcholine from preganglionic and parasympathetic nerve fibres and thus negatively regulate the M3 receptor pathway (Karakiulakis and Roth, 2012). Post-synaptic M2 receptors, which are also expressed in airway smooth muscle cells, attenuate adenylyl cyclase activity and thus promote smooth muscle relaxation (Montuschi and Ciabattini, 2015). Since ipratropium is a non-selective anti-muscarinic agent, we used the ratio of M3/M2 (CHRM3/CHRM2) receptor gene expression in lungs to estimate the potential responsiveness to ipratropium. The details of the eQTL study have been previously published (Hao et al., 2012). Briefly, gene expression profiles were obtained, using a custom Affymetrix array, which contained 51,627 non-control probesets, on 1111 lung tissue samples from individuals, who underwent lung resection surgery for a variety of different clinical indications, at 3 centres (Laval University, University of British Columbia [UBC] and University of Groningen). After quality control, the data were filtered and normalized. Samples that did not pass quality control were discarded. The final dataset contained 964 samples ($n = 441$ females; 46% of cohort). On the Affymetrix array, the CHRM3 and CHRM2 genes were represented by 100154030_TGI_at and 100310990_TGI_at probesets, respectively. The lung tissue expression data are available at NCBI Gene Expression Omnibus repository (GEO, <http://www.ncbi.nlm.nih.gov/geo>) through accession number GSE23546.

2.5. Statistical Analyses

To compare the baseline characteristics between male and female subjects, we used Wilcoxon rank sum test for continuous variables and chi-squared test for discrete variables. Because ipratropium induces acute bronchodilation but does not modify the long-term decline in FEV₁ (Cheyne et al., 2015) to evaluate the possible differential effects of ipratropium in female versus male subjects, we restricted our analysis to subjects in the SIA group of LHS who came back for spirometry at 4 month post-randomization. We chose the 4 month time point because compliance with ipratropium was greatest at this follow-up (compared with all the subsequent follow-up) with a rapid decay in compliance by 1 year post-randomization (Cheyne et al., 2015). We compared the mean changes in post-bronchodilator FEV₁ from baseline to 4 months between men and women using a two sample *t*-test. A multiple variable linear model was then used to evaluate the effects of sex on the change of % predicted FEV₁ at 4 months from baseline values in subjects who were in the SIA group. In this model we adjusted the results for age, body mass index (BMI), smoking intensity (as defined by exhaled carbon monoxide) and pack-years of smoking and also included a sex-BMI interaction and sex-pack-years interaction terms. To determine the impact that sex had on FEV₁ changes over 5 years, we used linear models which were adjusted for smoking status at each annual visit. For the lung eQTL dataset, we compared baseline characteristics between females and males using Wilcoxon rank sum tests for continuous variables and chi-squared tests for discrete variables.

The difference in the ratio of M3/M2 receptor gene expression between females and males was conducted for each sub-cohort (Laval, UBC, Groningen) separately, using linear models adjusting for age and smoking status. The results were then combined using inverse variance weighted meta-analysis assuming a fixed effect model. All analyses were conducted using R version 3.2.1.

3. Results

3.1. Baseline Characteristics of LHS

The baseline characteristics of the LHS cohort according to sex are summarized in Table 1. While there were no sex-related differences in age or the percent predicted post-bronchodilator FEV₁ of the subjects, female participants had lower BMI's and reduced cigarette smoke exposure, as evidenced by lower pack-years of smoking and older age of smoking initiation. As reported previously (Kanner et al., 1994), female subjects had increased airway responsiveness to methacholine.

3.2. Spirometry Values at 4 Months Following Randomization

The subjects in the SIA and SIP groups returned to the clinical centres at 4 months post-randomization to undergo spirometry. The mean change in post-bronchodilator FEV₁ from baseline was 0.10 L in the SIA group versus −0.09 L in the SIP group ($p = 1.37 \times 10^{-164}$). When FEV₁ was expressed as a percent predicted value, the mean change was 3.32% in the SIA group versus −2.33% in the SIP group ($p = 3.66 \times 10^{-166}$).

The change in FEV₁ at 4 months from baseline values in female versus male subjects who were randomized to SIA is shown in Table 2. Overall, in the SIA group, the female subjects experienced a $4.85\% \pm 5.66\%$ increase in their predicted FEV₁ from baseline; whereas in male subjects, they experienced a $2.34\% \pm 5.50\%$ increase in their predicted FEV₁ from baseline ($p = 2.61 \times 10^{-19}$ which compares the female versus male FEV₁ responses to ipratropium). Based on the recommendations of the ATS/ERS Task Force, we defined a clinically relevant improvement in FEV₁ as a change in FEV₁ of 140 ml or greater from baseline values (Cazzola et al., 2008). Using this threshold, 44.1% of female subjects versus 37.4% of male subjects experienced a significant improvement in their lung function at 4 months with ipratropium (p

Table 1

Baseline characteristics of Lung Health Study Cohort according to sex at the time of randomization.

	Women (n = 2185)	Men (n = 3702)	p-Value
Age (years)	48.55 ± 6.56	48.42 ± 6.98	0.71
Body mass index (kg/m ²)	24.19 ± 3.98	26.37 ± 3.66	<0.001
Smoking (pack-years)	36.37 ± 16.63	42.86 ± 20.07	<0.001
Cigarettes per day	28.89 ± 11.79	32.68 ± 13.24	<0.001
Age at smoking initiation (years)	18.13 ± 3.92	17.09 ± 3.78	<0.001
FEV ₁ (litres) ^b	2.17 ± 0.36	3.08 ± 0.49	<0.001
Subjects in GOLD 1 grade	986 (45.13%)	1728 (46.68%)	0.26
Subjects in GOLD 2 grade	1198 (54.83%)	1970 (53.21%)	0.24
Bronchodilator response (% of baseline FEV ₁)	4.31 ± 5.35	4.27 ± 4.95	0.99
SIA	769 (35.19%)	1192(32.20%)	<0.05
SIP	706 (32.31%)	1256(33.93%)	0.21
PC 20 (mg/ml)	6.35 ± 5.44	8.62 ± 6.61	<0.001
O'Connor Slope ^a	−18.33 ± 28.25	−9.47 ± 19.35	<0.001

Data are presented as mean ± SD for continuous variable and number of subjects (% column totals).

Abbreviations: FEV₁, forced expiratory volume in 1 s; GOLD, Global initiative for chronic Obstructive Lung Disease; PC, provocation concentration at which there is 20% fall in FEV₁; SIA, special intervention + Atrovent; SIP, special intervention + placebo.

^a O'Connor Slope is defined as the percentage of decline of FEV₁ from the post-saline value to the value measured after the final methacholine dose administered divided by the final cumulative methacholine dose administered.

^b All values are post-bronchodilator.

= 0.0066). In contrast, 15.2% of female and 31.4% of male subjects experienced a reduction in FEV₁ with ipratropium. In those in the SIP arm, there was a slight reduction in FEV₁ from baseline ($-2.33\% \pm 5.84\%$) with male subjects experiencing a slightly greater fall in FEV₁ compared with female subjects (male $-2.75\% \pm 5.46\%$ versus female $-1.58\% \pm 6.38\%$, $p = 0.0001$) when FEV₁ was evaluated as a percent predicted value.

In the multivariable analysis where we evaluated the differential effects of ipratropium on % predicted FEV₁ at 4 months (Table 3), we found that there was a significant interaction between sex and BMI. In females, the bronchodilatory effect of ipratropium bromide was inversely related to the subject's BMI; whereas in males, BMI had little effect on ipratropium's bronchodilatory effect (Fig. 1).

3.3. Spirometry Values From Baseline Through Year 5 Following Randomization

Over 5 years, among individuals in SIA and SIP groups, fewer female than male subjects were able to become sustained quitters ($p = 0.010$). However, among continuous smokers, females smoked fewer cigarettes per day than male smokers ($p = 0.00004$). Despite these differences, the average percent predicted FEV₁ at year 5 was similar between males and females ($p = 0.42$), with a similar rate of % predicted FEV₁ change per year over these 5 years ($p = 0.06$). These data are summarized in Table 4. Although the use of ipratropium did not modify the rate of decline in % predicted FEV₁ over the 5 years, its effect on FEV₁ was significantly greater in females versus males until year 3 for those assigned to SIA (Fig. 2).

3.4. Gene Expression for M2 and M3 Receptors in Lung Tissue

Because LHS did not collect lung samples for gene expression studies, we evaluated gene expression levels for the M2 (encoded by the CHRM2 gene) and M3 (encoded by the CHRM3 gene) muscarinic receptor in lungs of individuals who underwent lung resection surgery ($n = 436$ females and $n = 517$ males). The baseline characteristics of the lung

Table 2
FEV₁ changes from baseline to 4 months visit in male and female subjects in the special intervention + Atrovent (SIA) group.^a

	Women (n = 683)	Men (n = 1061)	p-Value
Change in FEV ₁ (litres)	0.13 ± 0.15	0.08 ± 0.22	<0.001
Change in FEV ₁ (% change from baseline)	6.00 ± 7.51	2.94 ± 7.53	<0.001
Change in FEV ₁ (change in % predicted FEV ₁)	4.85 ± 5.66	2.34 ± 5.50	<0.001
Individuals who experienced a 140 ml or greater increase in baseline FEV ₁	301 (44.07%)	397 (37.42%)	<0.01

^a All values are post-bronchodilator.

eQTL cohort are shown in Supplementary Table 1. Female subjects were slightly younger and had lower BMI, and smaller pack-years of smoking exposure than male subjects. There was increased lung gene expression of M3 receptor relative to the M2 receptor in female versus male lungs (meta-analysis p value of 6.86×10^{-8} adjusted for age and smoking; Fig. 3). When M3 receptor expression in lung was evaluated in isolation, there was greater M3 receptor expression in female versus male lungs (meta-analysis p value = 0.0047; Supplementary Fig. 1) though adjustments for age and smoking abolished these differences (p = 0.14).

4. Discussion

Despite the high burden of COPD in women, the current therapeutic strategies for COPD have been derived from clinical trials that have largely recruited male subjects (Vogelmeier et al., 2017). Using data from the Lung Health Study (LHS), which was made up of nearly 40% female subjects, we found that on average women experienced a larger bronchodilatory benefit of ipratropium compared with male subjects. Significantly, these effects were modified by BMI such that females in the lowest BMI categories experienced the largest benefits. At 4 months of therapy, 44% of the female subjects demonstrated a significant improvement in FEV₁ (defined by an increase of 140 ml or more in their FEV₁ from baseline) from ipratropium; whereas only 37% of the male patients met this threshold.

Our findings are consistent with the evolving concept that for the same cigarette exposure, female COPD patients have a greater burden of airway disease than male patients and that this risk may be modified by body mass index and presence of comorbidities (Dransfield et al., 2007; Cazzola et al., 2011; Cazzola et al., 2013). For instance, in patients undergoing lung volume reduction surgery, female lungs demonstrated greater wall thickening in small airways (<2 mm in size) than male COPD lungs (Martinez et al., 2007). These changes appear to occur early in the disease process, as using optical coherence tomography, we showed previously that female smokers have thicker airway walls than male smokers even when their FEV₁ was within the normal range (Tam et al., 2016). These human findings have been supported by data in murine models of COPD where exposure to 6 months of cigarette smoke resulted in significantly greater airway disease in female compared with male mice (but no significant differences in the extent of emphysema) (Tam et al., 2016). The mechanisms responsible for sexual dimorphism of COPD are largely unknown. In animals, removal of ovaries in female mice completely abolished the sex-related differences in the airway disease induced by cigarette smoke, suggesting that

female sex hormones may be involved in the process. However, it should be noted that there are translational limitations to animal studies in COPD, which have been previously highlighted (Churg et al., 2011). Thus, these data should be interpreted cautiously. Notwithstanding, in LHS and other studies (Langdeau et al., 2009; Tantisira et al., 2008), it has been shown that female smokers have increased airway responsiveness compared with male smokers, which may be related to their reduced airway geometry or increased small airway inflammation (Cohen et al., 2008; Sheel et al., 2009) and female COPD patients are more likely to have asthma overlap (Sin et al., 2016), which may increase airway responsiveness to bronchodilators (Montuschi et al., 2014). Supporting these data, animal experiments have shown that female mice compared with male mice demonstrate greater levels of bronchial responsiveness and airflow limitation related to viral infections in the respiratory tract (Larcombe et al., 2011). In female mice, bronchial reactivity increases with age; whereas in male mice, age plays no role (McKenzie et al., 2010). Whether these sex-related differences in airway reactivity explain differential responses to bronchodilators in COPD is not known.

Ipratropium bromide induces bronchodilation by reducing cholinergic tone in the airway smooth muscles by competitively binding to the muscarinic (M) acetylcholine receptors in the airways (Gross, 1988). Although ipratropium is a non-selective anti-muscarinic agent, its effect in the lungs is mediated largely through the M3 receptor, which upon activation causes smooth muscle contraction (Montuschi et al., 2013). The M2 receptor, on the other hand, auto-regulates acetylcholine (ACh) signaling by inhibiting ACh release by preganglionic and parasympathetic neural tissue (Alagha et al., 2014), though in the post-synaptic neural tissue, its stimulation may promote airway smooth muscle relaxation (Montuschi and Ciabattini, 2015). In lung tissues, we observed a modest upregulation of M3 receptor and most importantly a significant increase in M3 gene expression relative to M2 receptor expression in the lungs of female compared with male subjects, raising the possibility that there may be sexual dimorphism in the expression of these receptors in the airways that may modulate therapeutic responses to ipratropium. Additional studies will be required to validate these early observations.

We also observed a significant interaction between sex and BMI such that in females the bronchodilatory effects of ipratropium were inversely associated with BMI; whereas in males, BMI did not appear to modify this effect. Although there is a scarcity of studies that have evaluated the potential modifying effects of BMI on bronchodilatory responses in COPD, in asthma, obesity has been shown to reduce acute bronchodilator responses to short-acting beta-2 agonists (McGarry et al., 2015). The molecular basis of these observations remains elusive and further studies will be required to fully understand the complex relationship between sex and body mass in COPD.

There were limitations to the study. Firstly, ipratropium is a non-selective anti-muscarinic agent and is a relatively weak bronchodilator. This, the findings may not be generalizable to the newer more selective M3 inhibitors such as tiotropium, which are commonly used in the community and are generally more efficacious than ipratropium (Cheyne et al., 2015). Using the UPLIFT (Understanding the Potential Long-term Impact of Tiotropium) data, Tashkin et al. data showed that there were no significant differences in the responses to tiotropium, a more selective M3 inhibitor, between men and women with respect to FEV₁ decline

Table 3
Risk factors associated with significant changes in percent predicted FEV₁ from baseline to 4 months in subjects who were assigned to ipratropium bromide.

	Risk estimate	95% CI	p-Value
Sex (male versus females)	-5.50	(-9.31, -1.68)	<0.01
Age (per 1 year increase)	-0.07	(-0.12, -0.03)	<0.01
BMI (per 1 kg/m ² increase)	-0.14	(-0.25, -0.03)	<0.05
CO (per 1 ppm increase)	0.02	(0.01, 0.04)	<0.01
Smoking (per 1 pack-year increase)	0.04	(0.01, 0.07)	<0.01
Sex × BMI	0.15	(0.00, 0.30)	<0.05
Sex × pack-years	-0.02	(-0.05, 0.01)	0.233

Abbreviations: BMI, body mass index; CI, confidence interval; CO, carbon monoxide.

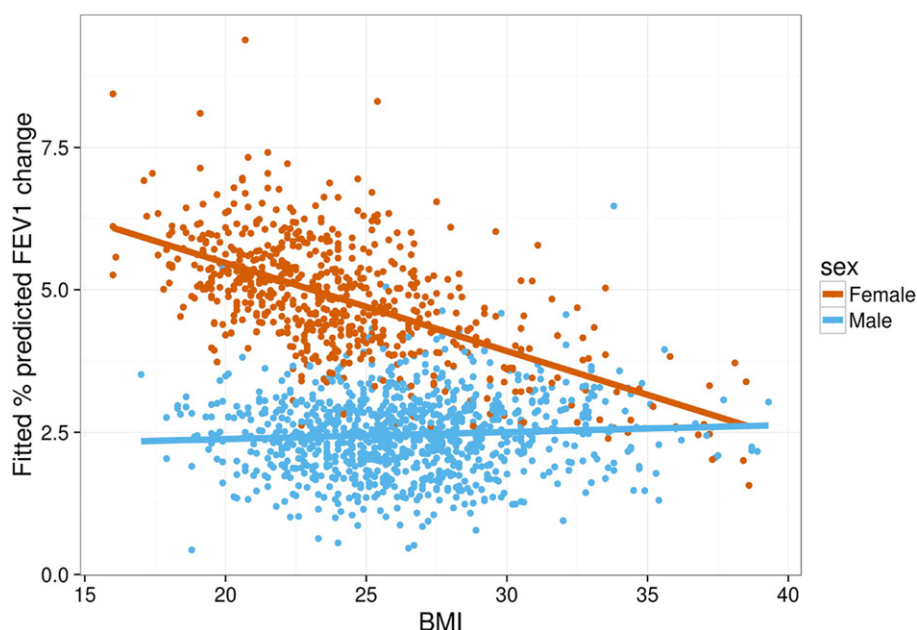


Fig. 1. The differential impact of body mass index in men and women on the bronchodilatory effects of ipratropium bromide over 4 months. The plot is a set of fitted lines based on a multivariate regression analysis in which age, sex, BMI, smoking have been adjusted. P for interaction = 0.044.

over 4 years (Tashkin et al., 2010). However, this study may have lacked sufficient power to adequately address this issue because only 25% of the cohort were female patients and 40% of patients (and more females than males, 45% versus 40%) discontinued the study prematurely. Secondly, although we observed significant sex-related differences in FEV_1 at 4 months and at 1 and 2 years of follow-up, these differences were largely lost beyond this time point. One likely explanation for this observation is the reduction in compliance with ipratropium over the 5 years of follow-up. Whereas the mean use of ipratropium was 4.4 puffs per day at 4 months of follow-up, by year 1, it was 3.67 puffs per day and by year 5, it was <3 puffs per day (Kanner et al., 1994). The progressive reduction in compliance to the medication likely attenuated the sex-related differences in FEV_1 related to ipratropium over time. Thirdly, while FEV_1 is an important endpoint for assessment of therapeutic efficacy of bronchodilators, there are other patient-centered outcomes such as exacerbation that are also relevant in their therapeutic assessment. LHS was powered on FEV_1 decline and as such robust data on exacerbations or health status were not available. Furthermore, LHS did not perform computed tomographic (CT) scanning and as such the effects of COPD phenotypes (emphysema versus small airways disease) on therapeutic responsiveness could not be assessed. Fourthly, LHS recruited individuals with mild to moderate COPD. Thus, the findings of this study may not be applicable to patients with severe or very severe COPD. It should also be noted that LHS was conducted in the 1990's when smoking rates were significantly higher and COPD management was significantly different than the current standards.

We also noted in this study that females on average smoked fewer cigarettes than male patients. Since cigarette smoking induces a persistent inflammatory response in the lung that leads to tissue injury and dysfunction, the sex-related differences in therapeutic responses to ipratropium may in part be related to differences in smoking behaviour between men and women (Malerba and Montuschi, 2012). Moreover, the lung samples were obtained from patients who underwent lung resection surgery, which may limit the generalizability of the results. Because the two studies (LHS and the Lung eQTL studies) were conducted in different subjects with different clinical characteristics, their results are not directly comparable.

The burden of COPD is rapidly growing in the female population. The findings of the present study indicate that female COPD patients may be more responsive to bronchodilators (in terms of FEV_1) and this effect is modified by body mass index. In an era of precision medicine, there is compelling and pressing need for future therapeutic trials to include sufficient numbers of female patients to ensure generalizability of findings to both men and women and to enable sex and gender based assessment of therapeutic responsiveness to novel therapies in COPD.

Funding Sources

This study was funded by the Institute of Gender and Health (the Canadian Institutes of Health Research). The Lung Health Study was funded by the US National Heart, Lung and Blood Institute and the

Table 4
Smoking behaviour and FEV_1 changes over 5 years in men and women.

	Women (n = 1315)	Men (n = 2187)	p-Value
% predicted FEV_1 at year 5 ^a	76.20 ± 11.89	75.78 ± 12.10	0.42
Change in FEV_1 from baseline (% of baseline)	−7.77 ± 10.01	−6.76 ± 10.16	<0.01
Change in % predicted FEV_1 from baseline (% of baseline)	−2.87 ± 10.50	−3.42 ± 10.55	0.06
Sustained quitters at year 5	262(19.92%)	519(23.72%)	<0.01
Intermittent quitters at year 5	454(34.52%)	659(30.13%)	<0.01
Continuous smokers at year 5	576(43.80%)	945(43.21%)	0.76
Mean number of cigarette smoked per day at year 5 (for continuous smokers)	19.90 ± 10.54	22.64 ± 13.58	<0.001
Exhaled CO levels (for continuous smokers; parts per million)	24.54 ± 20.06	25.14 ± 12.83	<0.05
Cotinine levels (for continuous smokers; ng/ml)	322.04 ± 166.94	353.44 ± 211.11	0.35

^a All FEV_1 values are post-bronchodilator.

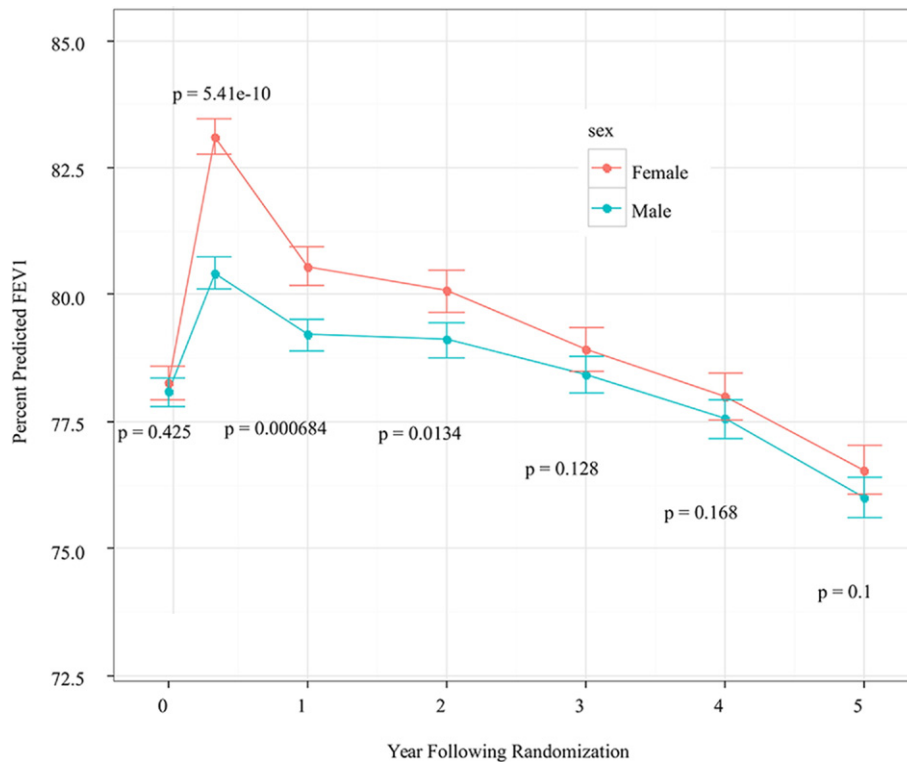


Fig. 2. Effects of Ipratropium Bromide on Percent Predicted FEV₁ over 5 years in male and female subjects in the Lung Health Study. Data are shown as mean plus minus SE. P values have been adjusted for age and smoking status for each time point comparisons.

Lung eQTL Study was funded by Merck. DDS holds a Tier 1 Canada Research Chair in COPD.

None of the funders played any role in the design of the study, data analysis or interpretation.

Lung eQTL data collection and analysis: PJ, YB, MVB, CAB, DCN, KH. Lung Health Study (LHS) data collection: DT, RB, JC. Statistical analyses: XL, MO, GZ, DDS. Wrote the manuscript: XL, MO, PDP, DDS.

Authors' Contributions

Conceived and designed the study: DDS, XL, MO, JML, PDP.

Conflicts of Interest

DCN is employed by Merck & Co. Inc.

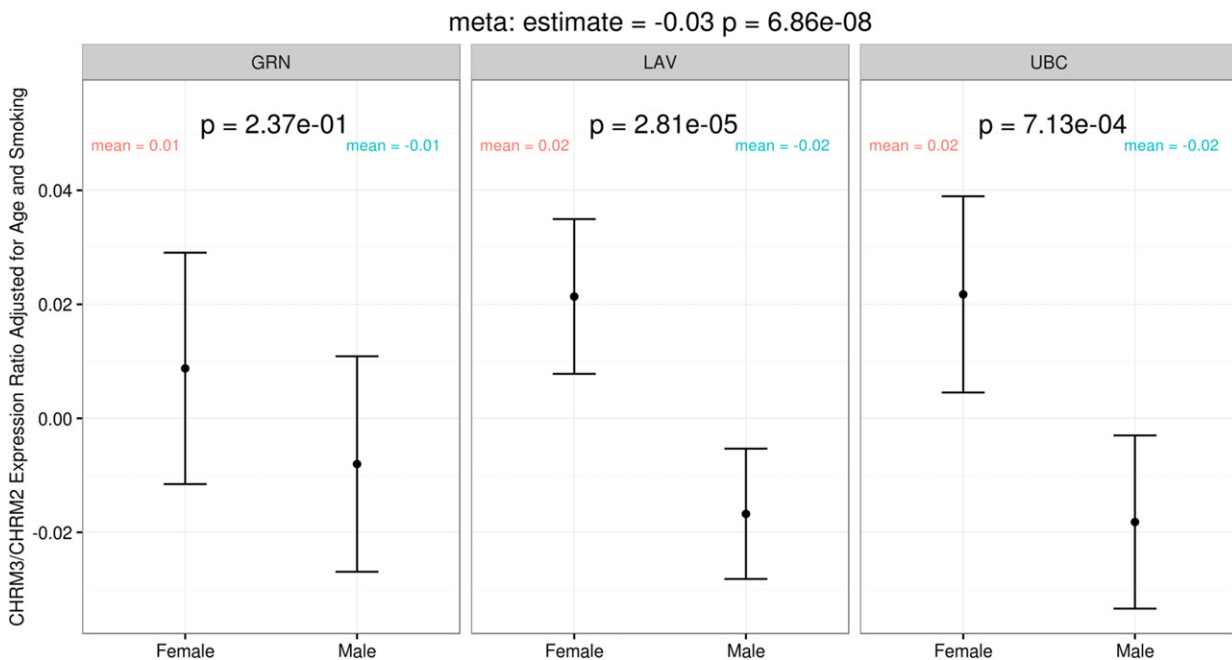


Fig. 3. The ratio in gene expression levels for M3 receptors relative to M2 receptors in lung tissue according to sex. Meta-analysis p-value = 2.99×10^{-8} . The p-values are adjusted for age and smoking status. Abbreviations: GRN, University of Groningen; LAV, Laval University; UBC, University of British Columbia.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.ebiom.2017.04.020>.

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