β2-Adrenergic Receptor (ADRB2) Gene Polymorphisms and Risk of COPD Exacerbations: the Rotterdam Study

(Online Supplementary Material)

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Supplemental methods

Baseline characteristics

BMI was calculated as weight divided by height squared (kg/m$^2$). Diabetes mellitus was defined as a fasting serum glucose concentration of $\geq 7.0 \text{ mmol/L}$ or a non-fasting serum glucose concentration of $\geq 11.1 \text{ mmol/L}$ or the use of blood glucose-lowering medications [1]. Hypertension was defined as a resting blood pressure above 140/90 mmHg or the use of blood pressure-lowering medication. The diagnosis of heart failure was based on follow-up using the medical records of the participants [2]. Coronary Heart Diseases (CHD) was defined as a compound outcome including fatal or nonfatal myocardial infarction or CHD mortality [2].

Systematic review

We conducted an extensive electronic literature search of Embase, Medline Ovid, and Cochrane Central using multiple search terms (Supplementary Table S1) to identify all articles investigating $ADRB2$ polymorphisms; rs1042713 and/or rs1042714 and/or their haplotypes and COPD exacerbations in patients exposed to $\beta_2$-agonists. Our literature search was restricted to studies published in English from inception until 30 September 2019. Additional potentially relevant articles were searched through article reference lists.
Review criteria and data extraction

We considered all original articles, excluding conference abstracts, editorials, short surveys, and animal studies. We did not set any limits on study design, sample size, location, or follow-up. Studies were included if they met the following three criteria;

(1) COPD patients exposed to inhaled short-acting $\beta_2$-agonists (SABA) and/or long-acting $\beta_2$-agonists (LABA) were eligible to be included in the review.

(2) The exposure variable of interest was $ADRB2$ polymorphisms; rs1042713 and/or rs1042714 and/or their haplotypes.

(3) The outcome of interest was COPD exacerbations. COPD exacerbation was defined as acute episodes of worsening symptoms requiring a course of systemic corticosteroid and/or antibiotics and/or hospitalization and/or emergency room visit.

The first author (LK) screened all studies from their titles and abstracts and excluded those that were not relevant. The full texts of potential papers were assessed independently by two authors (LK and KV). In case of heterogeneity across studies, the results of each study were reported individually.

Supplemental results

The literature search yielded 369 hits, of which 270 unique articles remained after excluding duplicates. Of these 270 articles, the title and abstract were reviewed and 236 articles were excluded (conference abstracts (26), editorials (10), experimental studies (5), short surveys (5) and as they were unrelated to the association between $ADRB2$ polymorphisms and treatment response to inhaled $\beta_2$-agonist in patient with COPD (190). We reviewed 34 full-text articles and 27 of these were excluded for the following reasons; review article (13), letter (1), focus on other SNPs in $ADRB2$ (3), focus on different outcomes (10). In total, three
clinical trials and four observational studies were withheld, but in the latter, not all of the included patients were on treatment with inhaled β₂-agonist (Figure S2).

Briefly, the three clinical trials that met inclusion criteria [3-5] were published between 2012 and 2014. The sample size ranged from 565 to 2,561. Two studies were multicentre, and another one was from the United States. One assessed the association between the SNPs and time to first COPD exacerbation using Kaplan-Meier curves and the log-rank test. [4] Rabe et al. found that patients with the Arg16Arg genotype and using salmeterol and inhaled corticosteroids (ICS) had a significantly lower risk of COPD exacerbations compared with Gly16Gly (p=0.0018) and Arg16Gly (p=0.0130) genotypes [4]. They found no significant differences in exacerbation risk between the genotypes of rs1042714. [4] Two other studies [3,5] assessed the association of the SNP(s) with the number of COPD exacerbations. One of them used Poisson regression to assess this association and while the other study described the distribution of the number of COPD exacerbation across the genotype categories of rs104213. They found no significant association between the SNPs and COPD exacerbations in COPD patients using LABA [3,5].

In our search, four observational studies [6-9] also evaluated the association of the SNP(s) with the number of COPD exacerbations. They were published between 2009 and 2019 and included patients from hospitals, medical centres, outpatient clinics, and the general population. Their sample size ranged from 61 to 5,219. However, not all of the included patients in these four observational studies were on treatment with inhaled β₂-agonist. The results of a recent observational study showed an increased risk of COPD exacerbations in carriers of Arg16 and Gln27 [9]. However, the proportion of COPD patients treated with LABA from the Copenhagen General Population Study was low (9.8 %) [9]. Due to differences in assessments and definitions of the outcome, this precluded a meta-analysis
with pooling of results. Therefore, we reported the findings separately for each study in Table 5 in the main text.
Supplementary tables and figures

Table S1: Search strategy per library

| Library          | Search Strategy                                                                 |
|------------------|---------------------------------------------------------------------------------|
| **Embase.com**   | ('adrb2 gene'/de OR 'adrb2 protein human'/de OR (adrb2 OR adrb-2):ab,ti OR (('beta 2 adrenergic receptor'/de OR 'beta adrenergic receptor'/de OR (((beta OR β OR beta2 OR β2) NEAR/3 adrenerg* NEAR/3 receptor*) OR ((beta OR β OR beta2 OR β2) NEAR/3 (adrenorecept* OR adrenocept* OR agonist*)):ab,ti) AND ('genetics'/exp OR 'genetic parameters'/exp OR 'genetic polymorphism'/exp OR genotype/exp OR 'genetic marker'/exp OR 'genetic association'/de OR 'genome-wide association study'/de OR (haplotype* OR polymorph* OR genetic* OR pharmacogenetic* OR snp OR genom* OR gwas):ab,ti))) AND ('chronic obstructive lung disease'/de OR (copd OR (chronic* NEAR/3 obstruct* NEAR/3 (lung OR pulmonar*)):ab,ti) AND [english]/lim |
| **Medline Ovid** | (ADRB2 protein, human.nm. OR (adrb2 OR adrb-2).ab,ti. OR ((Receptors, Adrenergic, beta-2/ OR Receptors, Adrenergic, beta/ OR (((beta OR beta2) ADJ3 adrenerg* ADJ3 receptor*) OR ((beta OR beta2) ADJ3 (adrenorecept* OR adrenocept* OR agonist*)):ab,ti.):ab,ti.) AND (exp Genetics/ OR Genetics.fs. OR exp Genetic Phenomena/ OR exp Genetic Association Studies/ OR (haplotype* OR polymorph* OR genetic* OR pharmacogenetic* OR snp OR genom* OR gwas).ab,ti.))) AND (Pulmonary Disease, Chronic Obstructive/ OR (copd OR (chronic* ADJ3 obstruct* ADJ3 (lung OR pulmonar*))).ab,ti.) AND english.la. |
| **Cochrane CENTRAL** | (((adrb2 OR adrb-2):ab,ti OR (((((beta OR β OR beta2 OR β2) NEAR/3 adrenerg* NEAR/3 receptor*) OR ((beta OR β OR beta2 OR β2) NEAR/3 (adrenorecept* OR adrenocept* OR agonist*)):ab,ti) AND ((haplotype* OR polymorph* OR genetic* OR pharmacogenetic* OR snp OR genom* OR gwas):ab,ti))):ab,ti) AND ((copd OR (chronic* NEAR/3 obstruct* NEAR/3 (lung OR pulmonar*)):ab,ti)) |
**Table S2:** Functional annotation of rs1042713 using the HaploRegv4.1

| Chr | pos (hg38) | LD (r²) | LD (D') | variant | Ref | Alt | EUR freq | Enhancer histone marks | DNAse | Motifs changed | Selected eQTL hits | GENCODE genes |
|-----|-------------|---------|---------|---------|-----|-----|---------|------------------------|--------|----------------|---------------------|---------------|
| 5   | 148819704   | 0.9     | 0.95    | rs35283004 | A   | G   | 0.38   | BLD, MUS               | GR,Maf | 2 hits         | 6.9kb 5' of ADRB2 |
| 5   | 148820281   | 0.81    | 0.92    | rs71582318 | T   | C   | 0.37   | BLD, SKIN              | Pou1f1,TATA | 6.3kb 5' of ADRB2 |
| 5   | 148821442   | 0.94    | 0.97    | rs12189018 | T   | C   | 0.38   | BLD                    | RXRA   | 2 hits         | 5.2kb 5' of ADRB2 |
| 5   | 148822166   | 0.94    | 0.97    | rs35019280 | AG  | A   | 0.38   | BLD                    | RXRA   | 2 hits         | 4.4kb 5' of ADRB2 |
| 5   | 148822926   | 0.93    | 0.97    | rs33910799 | AG  | A   | 0.38   | BLD                    | RXRA   | 1 hit          | 3.7kb 5' of ADRB2 |
| 5   | 148825014   | 0.97    | 0.99    | rs17778257 | A   | T   | 0.38   | 9 tissues              | SKIN   | 5 altered motifs | 1.6kb 5' of ADRB2 |
| 5   | 148826178   | 0.96    | 0.98    | rs12654778 | G   | A   | 0.38   | 38 tissues             | Foxp3,p53 | 4 hits         | 414bp 5' of ADRB2 |
| 5   | 148826877   | 1       | 1       | rs1042713  | G   | A   | 0.38   | 28 tissues             | 4 altered motifs | 3 hits        | ADRB2         |

*Pos,* position; *LD,* Linkage disequilibrium; *Ref,* reference; *Alt,* alternative; **EUR freq,** European frequency; **eQTL,** expression quantitative trait loci.
Table S3: Functional annotation of rs1042714 using the HaploRegv4.1

| chr | pos (hg38) | LD (r²) | LD (D’) | variant | Ref | Alt | EUR freq | Enhancer histone marks | DNAse | Motifs changed | Selected eQTL hits | GENCODE genes |
|-----|------------|---------|---------|---------|------|-----|----------|------------------------|--------|----------------|-------------------|---------------|
| 5   | 148819436  | 0.88    | 0.94    | rs4705059 | C    | T   | 0.59    | BLD, HRT, MUS           | HRT    | 5 altered motifs | 7.2kb 5’ of ADRB2 |               |
| 5   | 148819441  | 0.88    | 0.94    | rs4705060 | G    | A   | 0.59    | BLD, MUS                |        | 4 altered motifs | 7.2kb 5’ of ADRB2 |               |
| 5   | 148819679  | 0.9     | 0.96    | rs10078004| G    | A   | 0.60    | Mrg,NRSF                |        | BLD            | 6.9kb 5’ of ADRB2 |               |
| 5   | 148819882  | 0.9     | 0.96    | rs67339154| A    | G   | 0.60    | BLD                     |        | BLD, SKIN       | 6.7kb 5’ of ADRB2 |               |
| 5   | 148820448  | 0.94    | 0.97    | rs56330463| T    | C   | 0.59    | PPAR                   |        |                | 6.1kb 5’ of ADRB2 |               |
| 5   | 148820990  | 0.94    | 0.98    | rs2082382 | G    | A   | 0.60    | BLD, 38 tissues       | Foxo,Rad21 | 2 hits          | 5.6kb 5’ of ADRB2 |               |
| 5   | 148821037  | 0.97    | 0.99    | rs2082395 | A    | G   | 0.59    | BLD, 25 tissues       | 5 altered motifs | 2 hits          | 5.6kb 5’ of ADRB2 |               |
| 5   | 148821395  | 0.95    | 0.99    | rs9325120 | C    | A   | 0.58    | BLD                    |        | 4 altered motifs | 5.2kb 5’ of ADRB2 |               |
| 5   | 148821692  | 0.97    | 0.99    | rs11168066| C    | A   | 0.59    | BLD                    | Dmbx1,Otx2 | 2 hits          | 4.9kb 5’ of ADRB2 |               |
| 5   | 148821753  | 0.96    | 0.99    | rs11959615| T    | A   | 0.59    | BLD                    |        |                | 4.8kb 5’ of ADRB2 |               |
| 5   | 148821910  | 0.97    | 0.99    | rs35875547| AT   | A   | 0.59    | BLD, BRN               | 10 altered motifs | 4.7kb 5’ of ADRB2 |               |
| 5   | 148821922  | 0.97    | 0.99    | rs11958940| A    | T   | 0.59    | BLD, BRN               | NRSF,Zbtb3 | 4.7kb 5’ of ADRB2 |               |
| 5   | 148822006  | 0.97    | 0.99    | rs34064454| A    | G   | 0.59    | BLD, BRN               | AIRE,Pax-4 | 4.6kb 5’ of ADRB2 |               |
| 5   | 148823105  | 0.97    | 0.99    | rs11746634| C    | G   | 0.59    | ESC, BLD               | LUN-1,RORalpha1 | 3.5kb 5’ of ADRB2 |               |
| 5   | 148823238  | 0.97    | 0.99    | rs11168067| A    | G   | 0.59    | BLD                    | NRSF,Pitx2,SETDB1 | 3.4kb 5’ of ADRB2 |               |
| 5   | 148823373  | 0.95    | 0.99    | rs9325122 | C    | T   | 0.60    | BLD                    | HDAC2,Pou2f2,Pou3f3 | 3.2kb 5’ of ADRB2 |               |
| 5   | 148824199  | 0.97    | 0.99    | rs1432622 | T    | C   | 0.59    | BLD                    | 7 altered motifs | 2 hits          | 2.4kb 5’ of ADRB2 |               |
Table S3. Functional annotation of rs1042714 using the HaploRegv4.1 (cont’d)

| chr | pos (hg38) | LD (r²) | LD (D’) | variant | Ref | Alt | EUR freq | Enhancer histone marks | DNAse | Motifs changed | Selected eQTL hits | GENCODE genes |
|-----|------------|---------|---------|---------|-----|-----|---------|------------------------|--------|----------------|-------------------|---------------|
| 5   | 148824445  | 0.97    | 0.99    | rs1432623 | C   | T   | 0.59   | BLD, SKIN              | Nkx2   |                | 2.1kb 5’ of ADRB2 |               |
| 5   | 148825489  | 0.97    | 0.99    | rs2400707 | A   | G   | 0.59   | 12 tissues SKIN,SKIN   | HLF    | 8 altered motifs | 2kb 5’ of ADRB2  |               |
| 5   | 148825809  | 0.97    | 0.99    | rs2053044 | A   | G   | 0.59   | 5 tissues 35 tissues   | 8 altered motifs | 783bp 5’ of ADRB2 |               |
| 5   | 14882665   | 0.99    | 1       | rs11959427| C   | T   | 0.59   | BRN 52 tissues         | 11 altered motifs | 127bp 5’ of ADRB2 |               |
| 5   | 14882785   | 0.98    | 1       | rs1042711 | C   | T   | 0.59   | 35 tissues 6 altered motifs | 5’-UTR of ADRB2 |               |
| 5   | 148826812  | 0.98    | 1       | rs1801704 | C   | T   | 0.59   | BRN 37 tissues         | E2A,Sin3Ak-20,ZEB1 | 5’-UTR of ADRB2 |               |
| 5   | 148826910  | 1       | 1       | rs1042714 | G   | C   | 0.59   | 21 tissues             | GATA,PU.1 | ADRB2           |               |

Pos, position; LD, Linkage disequilibrium; Ref, reference; Alt, alternative; EUR freq, European frequency; eQTL, expression quantitative trait loci
**Figure 1:** Flowchart of participants

- **The Rotterdam study**
  - n = 14926

  Excluded:
  - Neither COPD nor Asthma (by 1-1-2011)
    - n = 12385

  COPD or Asthma (by 1-1-2011)
  - n = 2541

    Excluded:
    - Asthma (by 1-1-2011) n = 676
    - ACOS* (by 1-1-2011) n=194

  COPD patients (by 1-1-2011)
  - (n = 1671)

    Excluded:
    - No informed consent (n = 12)
    - No information on drug use and/or hospitalization (n =347)

  COPD patients with follow-up
  - (n = 1312)

    Excluded:
    - No genetics data (n = 259)

  COPD patients included in the analysis
  - (n = 1053)

* Asthma and COPD overlap syndrome
**Figure S2:** A flow chart describing the steps for including studies in the review

- **Embase.com** (n = 185)
- **Cochrane CENTRAL** (n = 10)
- **Medline Ovid** (n = 174)

Records retrieved
n = 369

Removed duplications
n = 99

Records after duplication removed
n = 270

Records excluded (n = 236)
1- Conference abstracts (n = 26)
2- Editorials (n = 10)
3- Experimental Studies (n = 5)
4- Short Surveys (n = 5)
5- Based on title and abstract (n = 190)

Full text articles assessed for eligibility
(n = 34)

Records excluded (n = 27)
1- Review (n = 13)
2- Comment letter (n = 1)
3- Different SNP/SNPs (n = 3)
4- Different outcomes (n = 10)

Included in systematic review
(n = 7)

- All participants were on inhaled β₂-agonist treatment (n = 3)
- Not all participants were on inhaled β₂-agonist treatment (n = 4)

All participants were on inhaled β₂-agonist treatment (n = 3)
Not all participants were on inhaled β₂-agonist treatment (n = 4)
Supplemental references

1. Ligthart S, van Herpt TT, Leening MJ, Kavousi M, Hofman A, Stricker BH, van Hoek M, Sijbrands EJ, Franco OH, Dehghan A. Lifetime risk of developing impaired glucose metabolism and eventual progression from prediabetes to type 2 diabetes: a prospective cohort study. The lancet. Diabetes & endocrinology 2016; 4: 44-51.

2. Leening MJ, Kavousi M, Heeringa J, van Rooij FJ, Verkroost-van Heemst J, Deckers JW, Mattace-Raso FU, Ziere G, Hofman A, Stricker BH, Witteman JC. Methods of data collection and definitions of cardiac outcomes in the Rotterdam Study. European journal of epidemiology 2012; 27: 173-185.

3. Bleecker ER, Meyers DA, Bailey WC, Sims AM, Bujac SR, Goldman M, Martin UJ. ADRB2 polymorphisms and budesonide/formoterol responses in COPD. Chest 2012; 142: 320-328.

4. Rabe KF, Fabbri LM, Israel E, Kogler H, Riemann K, Schmidt H, Glaab T, Vogelmeier CF. Effect of ADRB2 polymorphisms on the efficacy of salmeterol and tiotropium in preventing COPD exacerbations: a prespecified substudy of the POET-COPD trial. The Lancet. Respiratory medicine 2014; 2: 44-53.

5. Yelensky R, Li Y, Lewitzky S, Leroy E, Hurwitz C, Rodman D, Trifilieff A, Paulding CA. A pharmacogenetic study of ADRB2 polymorphisms and indacaterol response in COPD patients. The pharmacogenomics journal 2012; 12: 484-488.

6. Emeryk-Maksymiuk J, Emeryk A, Krawczyk P, Wojas-Krawczyk K, Milanowski J. Beta-2-adrenoreceptor polymorphism at position 16 determines the clinical severity of chronic obstructive pulmonary disease. Pulmonary pharmacology & therapeutics 2017; 43: 1-5.
7. Hussein MH, Sobhy KE, Sabry IM, El Serafi AT, Toraih EA. Beta2-adrenergic receptor gene haplotypes and bronchodilator response in Egyptian patients with chronic obstructive pulmonary disease. Advances in medical sciences 2017; 62: 193-201.

8. Vacca G, Schwabe K, Duck R, Hlawa HP, Westphal A, Pabst S, Grohe C, Gillissen A. Polymorphisms of the beta2 adrenoreceptor gene in chronic obstructive pulmonary disease. Therapeutic advances in respiratory disease 2009; 3: 3-10.

9. Ingebrigtsen TS, Vestbo J, Rode L, Marott JL, Lange P, Nordestgaard BG. β2-Adrenergic genotypes and risk of severe exacerbations in COPD: A prospective cohort study. Thorax 2019.