Association between the adrenoreceptor β2 gene and paediatric asthma severity – a study of the PACMAN cohort

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INTRODUCTION

Asthma is the most common chronic disease among children and is recognized as a public health problem [1]. In 2019, it was estimated that asthma affected approximately 334 million people worldwide [2].

It is a chronic lung disease characterized by inflammation and narrowing (of the tissue) of the airways. This process results in breathing difficulties and a triggering of other symptoms such as coughing, shortness of breath, chest tightness, and wheezing [3,4].

In addition to the narrowing of the airways, the symptoms are the result of the constriction of the muscles surrounding the airways and the extraordinary production of mucus from the airways, resulting in a decreased airflow [5].

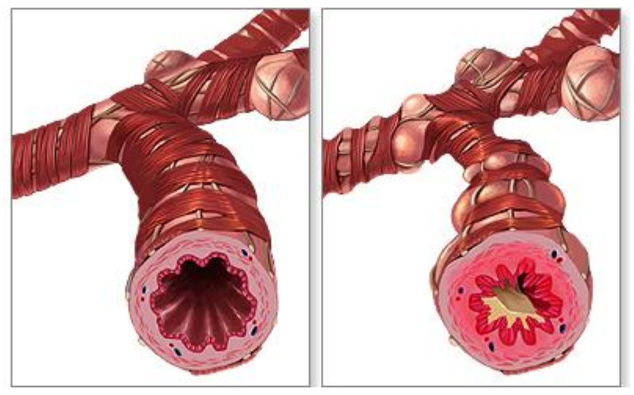

Figure 1: Normal and asthmatic bronchioles (Source: MedlinePlus).

Asthma prevalence and mortality have shown significant differences based on race and ethnicity. This includes aspects such as lung capacity and response to treatment, but due to the immense human phenotypic and genetic variation, specific racial and ethnic variation in genetic risk factors, among others, must be considered [6].

This project focused on a specific genetic variation in the **Adrenoreceptor-Beta 2 (β2) (ADRB2) gene**. This gene encodes the β2-adrenergic receptor (β2 adrenoreceptor) and has a significant influence in several systems in the human body [7].

The response to β2 agonists and the performance of the receptor has shown to differ according to the polymorphisms of the receptor. The **Arg16Glu polymorphism of the β2 adrenoreceptor gene** (ADRB2) has been associated with clinical drug response and asthma exacerbations [8].

OBJECTIVES

The main objective was to assess the association between asthma severity and the **Arg16Glu polymorphism of the β2 adrenoreceptor gene** (ADRB2).
The PACMAN cohort data was considered and through this, the dispensing of oral corticosteroids (OCS) prescriptions served as a proxy of the disease severity since corticoids are commonly used in uncontrolled asthmatic states (exacerbations).

For this purpose, **Count Regression Models** were used, according to the behavior of the data. In order to better understand whether the gene functions as a distinguishing factor in treatment response (and therefore in worse disease control), or not, a **genetic approach** on the matter is fundamental.

MATERIALS

The data used during the project is referent to a retrospective study entitled: **Pharmacogenetics of Asthma medication in Children: Medication with Anti-inflammatory effects** (PACMAN) - performed in the Netherlands.
Pharmaceutical records were linked to the previously mentioned data.

Through the Anatomical Therapeutic Chemical classification (ATC) codes provided in the pharmaceutical records, the asthmatic medication was resumed into six categories. These categories convey different needs of the patient and allow the assessment of disease condition and control.
They were classified as:

- Inhaled corticosteroids (ICS);
- Long-acting beta agonists (LABA);
- Long-acting beta agonists and inhaled corticosteroids (LABAICS);
- Leukotriene-receptor antagonists (LTRA);
- Oral corticosteroids (OCS);
- Short-acting beta agonists (SABA).

METHODS

The response variable was formulated as the **number of the child's OCS pharmaceutical prescriptions**.
Upon investigating the distribution of this variable, it was found that it was highly skewed by a predominance of **zero value** observations, as depicted in the figure 2 below.

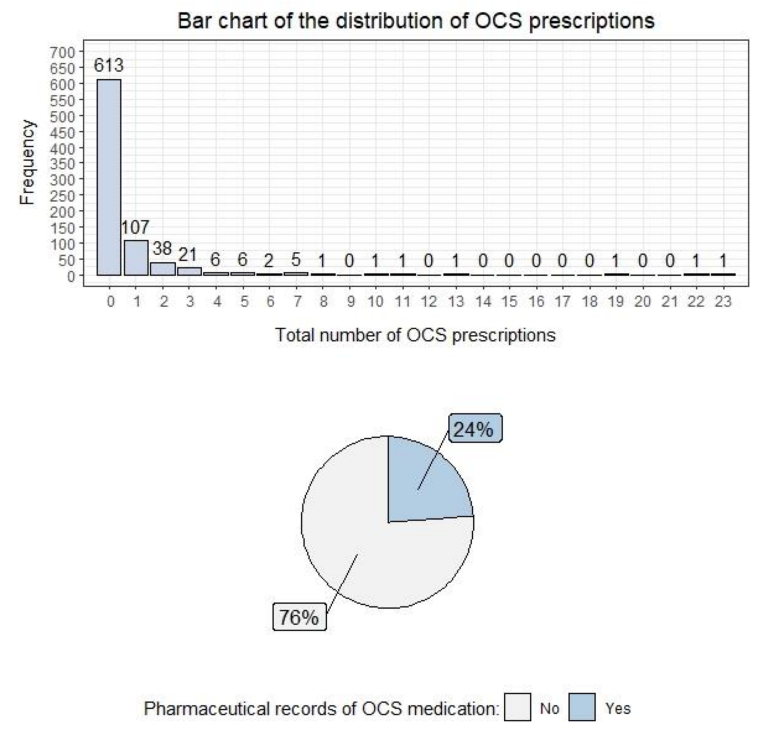

Figure 2: Bar and pie chart representations of the number of OCS prescriptions in the database.

Regression models were used to assess the association between the number of OCS and other variables of interest.

Models of the class of the Generalized Linear Models (GLM) for count data with excess of zeros:

- **Zero-inflated** models
- **Hurdle** models,

Considering a **Negative Binomial** distribution to account for **overdispersion**. Each model included an offset, to account the exposure.

The classes of models used to answer the research question had the following interpretations of the zero values (that accounted for 76% of the observed values of the response variable):

Hurdle Negative Binomial model

- If the child has no records of OCS intake so far, then this value will always remain the same.

Zero-Inflated Negative Binomial

- Controlled asthma state, no need to intake OCS.
- Asthma not fully controled, but the child was not prescribed OCS.

Both models included the same covariates:

- Age in years;
- Gender;
- Consumption of LTRA;
- Years of follow-up time in the study;
- Polymorphism.

The **additive genetic model** was used, which assumes that the affect of each allele is additive. They are based on the concept that the effect of each allele is proportional to the number of copies of that allele.

The variable regarding the polymorphism was then formulated as the **number of copies of the minor allele**.

The minor allele in this data, was the "A" allele.

RESULTS

I. Hurdle Negative Binomial model:

Regarding the count and zero hurdle components, the model equation for each component is given by:

$$\hat{\mu}_i = \exp(-10.55 - 0.27\mathrm{Polymorphism_i} + 1.04\mathrm{Gender_{Male_i}} + 0.07\mathrm{Age_i} + 0.93\mathrm{LTRA_{Yes_i}} - 0.30\mathrm{Gender_{Male}} \times \mathrm{Age_i}) \times \mathrm{YearsOfFollowUpTime_i}$$

$$\hat{p}_i = \frac{\exp(-2.23 + 0.31\mathrm{Polymorphism_i} - 0.03\mathrm{Age_i} + 0.12\mathrm{YearsOfFollowUpTime_i})}{1 + \exp(-2.23 + 0.31\mathrm{Polymorphism_i} - 0.03\mathrm{Age_i} + 0.12\mathrm{YearsOfFollowUpTime_i})}$$

$$(i = 1, \cdots, n = 805;\ 0 \le \hat{p}_i \le 1;\ \hat{\mu}_i > 0)$$

Table 1: Analysis of the HNB fitted model (only covariantes with significant association).

Covariate	IRR	95% CI (IRR)	P-value	Model component
Gender_Male	2.840	(0.989; 8.158)	0.052	Count component
LTRA_Yes	2.533	(1.225; 5.115)	0.010	
Gender_Male:Age	0.739	(0.558; 0.980)	0.035	
Polymorphism	1.363	(1.069; 1.736)	0.012	Zero-hurdle compoent
YearsOfFollowUpTime	1.128	(1.063; 1.196)	6.03E-05	

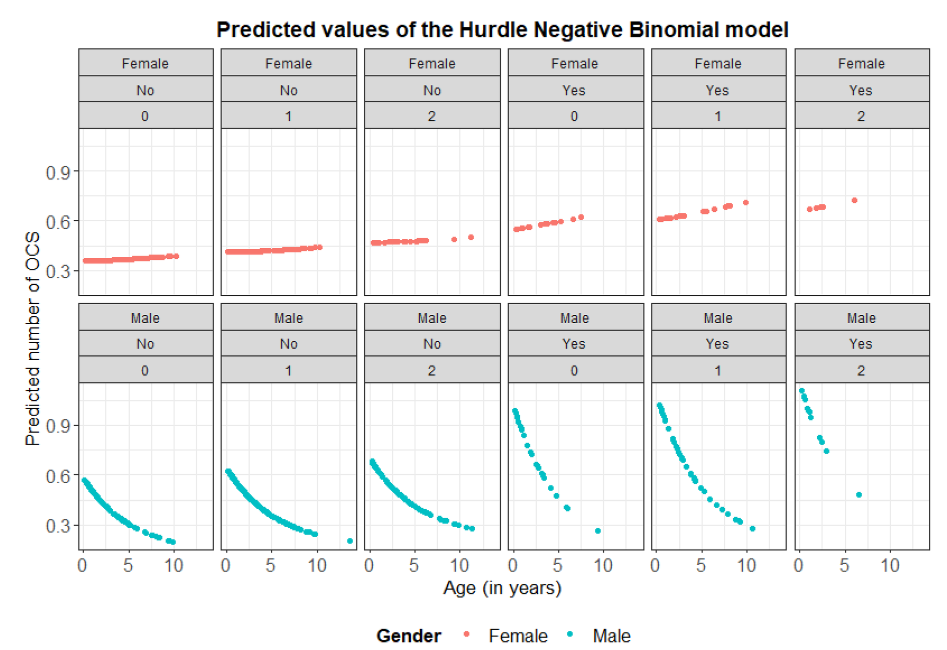

Figure 3: Predicted values of the Hurdle Negative Binomial model, given the observed data.

II. Zero-inflated Negative Binomial model:

Regarding the count and zero-inflation components, the model equation for each component is given by:

$$\hat{\mu}_i = \exp(-3.18 + 0.03\mathrm{Polymorphism_i} + 0.96\mathrm{Gender_{Male_i}} 0.06\mathrm{Age_i} + 0.81\mathrm{LTRA_{Yes_i}} - 0.21\mathrm{Gender_{Male}} \times \mathrm{Age_i}) \times \mathrm{YearsOfFollowUpTime_i}$$

$$\hat{p}_i = \frac{\exp(-13.23 - 5.56\mathrm{Polymorphism_i} - 3.44\mathrm{Age_i} + 1.23\mathrm{YearsOfFollowUpTime_i})}{1 + \exp(-13.23 - 5.56\mathrm{Polymorphism_i} - 3.44\mathrm{Age_i} + 1.23\mathrm{YearsOfFollowUpTime_i})}$$

$$(i = 1, \cdots, n = 805;\ 0 \le \hat{p}_i \le 1;\ \hat{\mu}_i > 0)$$

Table 2: Analysis of the ZINB fitted model (only covariantes with significant association).

Covariate	IRR	95% CI (IRR)	P-value	Model component
Gender_Male	2.613	(1.437; 4.749)	0.002	Count component
LTRA_Yes	2.240	(1.450; 3.461)	0.000	
Gender_Male:Age	0.811	(0.696; 0.945)	0.007	
YearsOfFollowUpTime	3.429	(0.922; 12.756)	0.066	Zero-inflation compoent

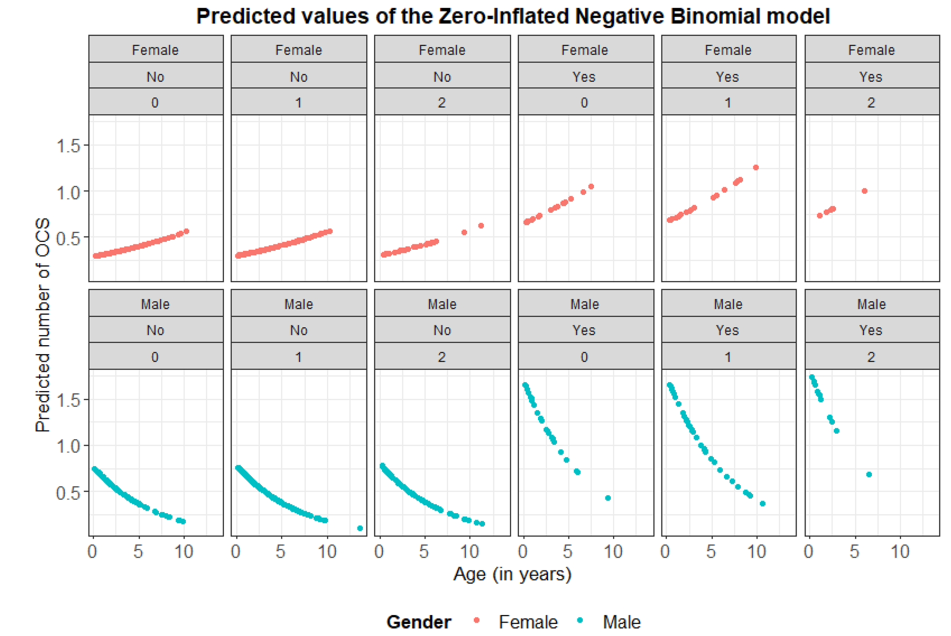

Figure 3: Predicted values of the Zero-inflated Negative Binomial model, given the observed data.

VALIDATION

To each of the models presented previously, the model's goodness-of-fit was evaluated through **rootgrams representations**, and the residual analysis was performed considering **Randomized Quantile Residuals**.

- The model's rootgrams revealed that the residuals hold small values, and do not exhibit any sort of visible pattern. In addition to this, no evidence was found that might suggested the models were under or over predicting;
- The randomized quantile residuals appeared to follow a Normal distribution, which sustained the hypothesis that both models were accurate to model the data.

These validation tools all pointed to a conclusion of well-adjusted models for the data.

CONCLUSIONS

- Univariate ZINB models where used, whilst considering the exposure time in the study (i.e, a longer time in asthma treatment), to characterize the children's overall consumption of the categorized drugs. They revealed that children consume, on average, consists of more ICS and SABA medications. The least "consumed" classes of medications are those that are needed in emergency situations (OCS) or poor control of the child's asthmatic condition (LTRA).

- In the regression analysis of the data, two types of models were considered to account the data's zero-values predominance and overdispersion.
- The first, the Hurdle Negative Binomial model showed a significant association between the children's OCS consumption and the number of the copies of the minor allele (in the zero-hurdle component). Other variables showed association with the response variable, namely the follow-up time (in the zero-hurdle component), the consumption of LTRA medication and the interaction between the children's age and gender. This model exhibited values of 1430.1 and 1481.7 regarding the Akaike and Bayesian Information Criterion, respectively.
- The remaining model, the Zero-Inflated Negative Binomial model did not show any significant association between the children's OCS consumption and the number of the copies of the minor allele. Other variables showed association with the response variable, namely the follow-up time (in the zero-inflation component), the consumption of LTRA medication and the interaction between the children's age and gender (as in the hurdle negative binomial model). This model exhibited values of 1433.4 and 1485.0 regarding to the Akaike and Bayesian Information Criterion, respectively.

- Comparison tests were performed to assess whether a specific model fitted the data more accordingly than the other. According to the Vuong's Test, there is no significant difference on the quality of the fit of both models.
However, according to the rule-of-thumb proposed by Adrian Raftery (1995), it is indicated that there is a positive evidence in favor of the model with the smaller BIC – this being the additive HNB model.
- Exploratory data analysis revealed that children with larger values of follow-up time, have a higher diversity of prescriptions of categorized asthmatic medication. Given the poor control of this disease that is still verified nowadays, the model that according to the literature is more appropriate, would be the Zero-inflated.

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