Personalized ADRB2 genotype-guided treatment to improve quality of life in pediatric asthma patients

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Abstract

Background

Asthma is the most common respiratory disease in children. Despite the use of medication, many children continue to experience uncontrolled asthma symptoms with a burden of quality of life (QoL) as a consequence. The Global Initiative for Asthma (GINA) guidelines suggest that children who continue to experience asthma symptoms at step 2 of the asthma treatment, should receive a long-acting β-agonist (LABA) in addition to an Inhaled Corticosteroid (ICS) or an ICS at double the dosage. Treatment decisions are not tailored to the individual patient, rather a one-size-fits-all principle is applied. The use of personalized treatment may reduce the incidence of non-responders. It is known that a variation in the gene coding for the adrenoceptor-β2 (ADRB2) results in an impaired response to a LABA. Therefore, children who carry the risk variant may benefit more from an ICS compared to a LABA.

Aim

This study investigates whether ADRB2-genotype guided treatment, in children with asthma on step 2 of the GINA guidelines, results in an improvement in the Pediatric Asthma (Caregiver) Quality of Life questionnaire (PA(C)QLQ) compared to the standard care.

Method

The PUFFIN study (Pharmacogenetics Use For Further Treatment Improvement in Children) is a multicenter, international, double-blind clinical trial performed in the Netherlands and Switzerland. 102 patients (age 6-18 years) with an asthma diagnosis on step 2 of the GINA guidelines with uncontrolled asthma symptoms were enrolled. Patients were randomized (1:1) to either the genotype-guided treatment or the standard treatment. Saliva samples were collected to determine the genotype. Patients in the genotype-guided arm received either LABA (Gly16Gly) or a double dosage ICS (Arg16Gly/Arg16Arg) based on their genotype and the control arm was randomized (1:1) to either LABA or ICS. The study duration was 6 months. The primary endpoint was the change in overall score in PA(C)QLQ. Results were analyzed with a Mixed Model Repeated Measure (MMRM) analysis. Secondary endpoints were changes in the QoL-score of the domains and the self-reported adherence according to the MARS-5 questionnaire.

Results

The genotype-guided treatment showed no difference in the improvement of QoL compared to the standard care (-0.079, 95%CI -0.348-0.226; p=0.608). Moreover, no difference in activity limitation score (0.017, 95% CI -0.387-0.421; p=0.933), emotional function score (-0.123, 95% CI -0.379-0.133; p=0.341) and symptom score (0.209, 95% CI -0.687-1.104; p=0.634) were seen. The results of the self-reported adherence showed no significant difference between the intervention arm and the control arm.

Conclusion

In this study, no difference in improvement in QoL was seen between the genotype-guided arm and the standard care arm. However, the direction of the results indicates that genotype-guided treatment may positively affect the QoL in pediatric asthma patients. This potential benefit warrants further exploration with a larger sample size or meta-analysis. Currently, harmonization with the PACT study [14] is being worked on.

List of abbreviation

ACT	Asthma Control Test
ADRB2	Adrenoceptor-β2
UMC	University Medical Center
FEV1	Forced expiratory volume in 1 second
FVC	Forced Vital Capacity
GCP	Good Clinical Practice
GINA	Global Initiative for Asthma
HRQOL	Health-Related Quality Of Life
ICS	Inhaled Corticosteroids
IQR	InterQuartile Range
LABA	Long-Acting β-Agonists
LTRA	Leukotriene receptor agonists
MARS	Medication Adherence Report Scale
MMRM	Mixed Model Repeated Measures
SABA	Short-Acting β-agonists
PACT	Personalized Medicine for Asthma Control
PA(C)QLQ	Pediatric Asthma (Caregiver's) Quality of Life Questionnaire
PROMs	Patient-Related Outcomes
PUFFIN	Pharmacogenetics Use For Further treatment Improvement in children
SNP	Single Nucleotide Polymorphism

Introduction

Asthma is the most common respiratory disease in children. Depending on the onset and definition of asthma, approximately 8 to 10% develop asthma. The most common symptoms are wheezing, shortness of breath, chest tightness, and coughing. Despite treatments available to lessen these symptoms, in many children asthma remains uncontrolled. [1][2]. The treatment follows a step-wise approach. Children on step 2 of the GINA guidelines with uncontrolled asthma will continue to go to step 3 which is either adding a LABA or doubling the dosage of ICS (figure 1). Another option for step 3 is LTRA. However, this addition is not a commonly chosen treatment among respiratory pediatricians in the Netherlands. [3] Treatment decisions are not always as straightforward. It is not a one-size-fits-all principle. Thus, uncontrolled asthma symptoms may remain despite the given treatments.

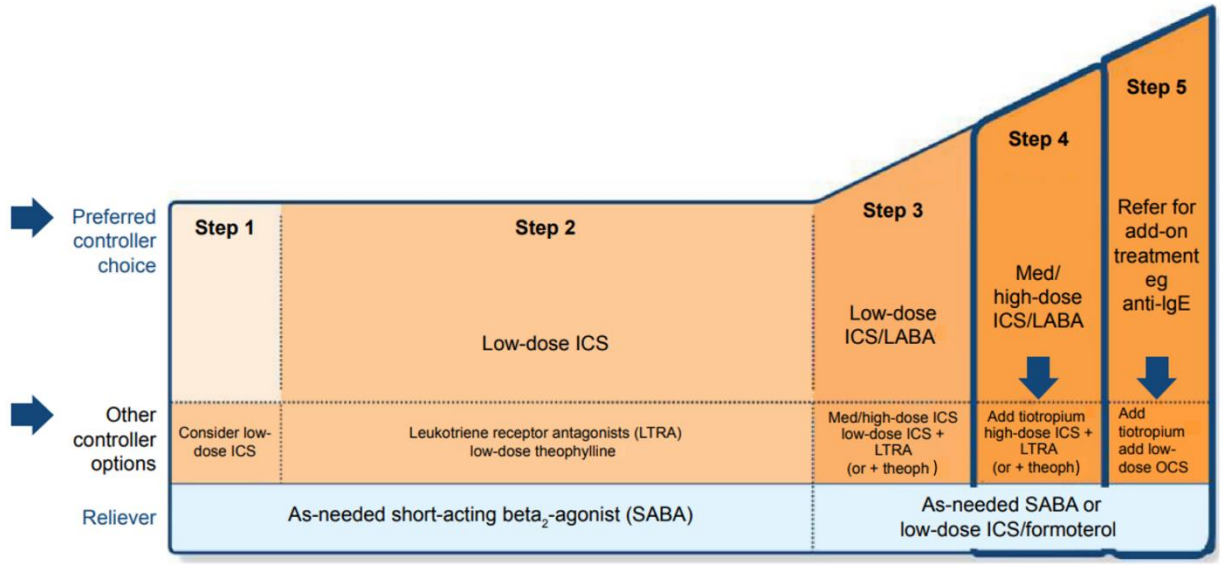

Figure 1: GINA guidelines for asthma management. Adapted from: [4]

The sustaining and uncontrolled asthma symptoms in children can have a substantial effect on the HRQOL. [5] HRQOL is multidimensional and includes multiple domains regarding physical, mental, and social well-being. Domains that can be distinguished are symptoms (e.g. wheezing, shortness of breath, and coughing), emotional function (frustration, anger, anxiety, and fear) and activity limitation (sleeping, social activities and performances in school). Therefore many aspects need consideration when trying to obtain a full picture of the health of the children (PROMs) [8]. It is known that there is an association between the QoL of children with asthma and family functioning. Moreover, it is found that children with asthma have a significantly poorer QoL compared to children without asthma [6][7]. In the end, an impaired QoL can influence the daily life of both the child and the family. Medical costs due to hospitalizations and absenteeism have a significant economic burden and are even expected to increase [8]. Additionally, a higher level of self-efficacy is associated with a better quality of life in both children and caregivers [9].

It is recognized that asthma is a highly heterogeneous disease. A way to obtain personalized medicine to tackle severe asthma symptoms is pharmacogenomics. Pharmacogenomics is the study of how genes affect and influence treatment responses to medication [10]. An example related to the importance of genetics while treating children with severe asthma is related to the SNP of the gene coding for the adrenoceptor-β2 (ADRB2) (wild type: Gly16Gly). Variations of this gene are associated with an impaired response to LABA. These variations (Arg16Gly and Arg16Arg) occur due to an arginine-16 polymorphism where the glycine at the 16th place is replaced by arginine. Around 17% of the population has the homozygous Arg16Arg variant and around 50% has the heterozygous Arg16Gly variant. This implies that only 33% of the population has the wild type (Gly16Gly) variant and only these patients would have an effective response to LABAs. [9] The underlying mechanism involves a greater susceptibility to agonist-induced down-regulation and uncoupling of β2-receptors in the airways. Therefore, fewer receptors are available to be targeted and this results in an impaired response to LABAs [11].

Studies have investigated the arginine-16 polymorphisms in patients and found that patients with the homozygous Arg16Arg variant have a significantly impaired and poorer response to salmeterol and more chance of developing exacerbations than patients with the wildtype [12][13]. Previous studies also found a significant difference in QoL when genotype-guided treatment is given with either LABA or LTRA based on the genotype (PACT study) [14]. Furthermore, a significant decrease in school absences was seen in children who received genotype-guided treatment regarding the ADRB2 risk variants [15]. These associations were specifically with LABA's and not with other medications targeting the β2-agonist such as Short Acting β-agonists (SABA). In the end, LABA is possibly not the first choice at step 3 of asthma management for children with an arginine-16 polymorphism and may benefit more from an ICS.

In conclusion, uncontrolled asthma has a vast impact on both the child and the family environment. A personal individualized approach based on polymorphism of ADRB2 could be a tool to further improve asthma treatment in children. This study aims to evaluate the influence of ADRB2-genotype-guided treatment on the quality of life, determined by the PA(C)QLQ, in children (age 6-18 years) with uncontrolled asthma compared to standard care. Data is used from the PUFFIN trial (NCT03654508). We hypothesize that children with ADRB2-genotype-guided treatment will experience a significantly better quality of life than children with standard care.

Method

Study design

The study was an international, multi-center, randomized controlled double-blind trial (PUFFIN; NCT03654508). Both patients and physicians were blind to the randomization processes. Participants were recruited from 3 academic hospitals and 9 non-academic hospitals in the Netherlands and 1 academic hospital in Switzerland at outpatient asthma clinics. The study duration for each patient was 6 months.

Study subjects

The study consisted of children (6-18 years) of either sex. Key inclusion criteria were a doctor's diagnosis of asthma (ever) based on the patient's history; ICS use ≥3 months before inclusion (supplementary table S1) at step 2 of the asthma treatment who required a step-up in asthma treatment; adequate use of low dose ICS; both caregiver(s) and the child needed to fill in the informed consent; the PA(C)QLQ questionnaire must be filled in and an adequate inhalation technique determined by the treating physician. Key exclusion criteria were active smoking; congenital heart disease; serious lung disease other than asthma and an ICU admission in the previous year. Full inclusion and exclusion criteria are provided in the supplemental methods, page 20.

Randomization strategy

In total 2 randomization steps were performed (figure 2). If eligible, patients were first randomized between the ADRB2-genotype-guided arm and the control arm using block randomization stratified per center (non-academic vs academic) with randomly chosen block sizes in a 1:1 manner. Within the control arm, the second randomization step was performed to determine whether the patient in the control arm received a double dosage ICS or a LABA. Within the genotype-guided arm, no randomization took place. The given treatment was based on the ADRB2-genotype: homozygote wild-type (Gly16Gly) received a LABA and heterozygote (Arg16Gly) and homozygote variant (Arg16Arg) received a double ICS dosage.

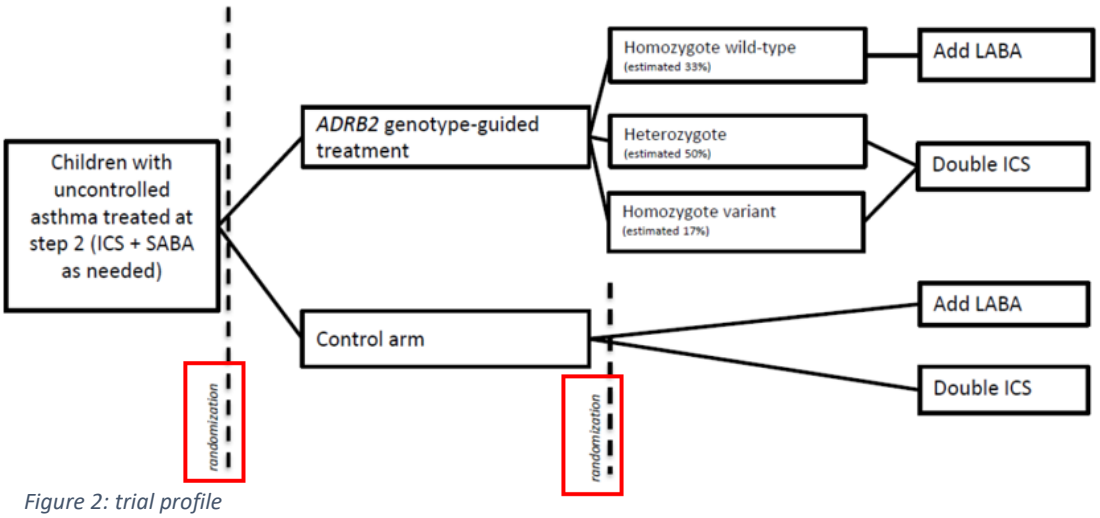

Figure 2: trial profile

The randomization strategy utilized in this study was block randomization with randomly chosen block sizes to ensure a balance in sample size and stratification per center to prevent uneven distribution of patients from non-academic and academic hospitals. Together this combination helped to even out the variability within the study population [16].

Study visits

During the first visit to the hospital at t=0 months, the patient was screened. This meant checking whether the patient met the in- and exclusion criteria. When the patient was eligible, both the caregiver(s) and the child had the opportunity to sign the informed consent. Only after both the caregiver(s) and the child signed the informed consent, the child continued with the study. The second and third visits to the hospital were respectively on t=3 and t=6 months. These visits were routine appointments for the patient in the hospital. At baseline, after 3 months and after 6 months the questionnaires were asked to be filled in. The research visits were performed by qualified professionals according to the Standard Operating Procedure.

If the asthma symptoms remain uncontrolled at t=3 according to the physician, the treatment regime will be adapted. A table of switches in treatment regimens is provided in supplementary table S2.

Questionnaires

Multiple questionnaires and questions were asked. For the primary endpoint, the questionnaire PA(C)QLQ was used. The PA(C)QLQ was used to assess the asthma-related quality of life. Children of the age of 12 years or older were asked to complete the PAQLQ. The parents of the children under the age of 12 were asked to complete the PACQLQ. The self-reported MARS questionnaire was used to assess medication adherence and was filled in by the parents. Other questions regarding comedication were asked during the first baseline visit 1.

Sampling

Samples were taken during the baseline visit at t=0. Saliva samples were taken regarding DNA isolation. The sample was sent to the Clinical Chemistry department of the Erasmus MC to execute genotyping of the ADRB2 gene. This was done within one week. The sample was genotyped with Illumina 500 (+duo) and Illumina 610-Quad Bead Chips. Genotypes were extracted for SNP rs1042713 (16Arg > Gly). The quality control procedures were applied. [17][18] Advise regarding the treatment will be based on the advice of the study coordinator.

Outcomes

Qualified professionals at the hospitals administered the PA(C)QLQ to the children and the caregivers during the visits to the hospital. Children and caregivers were asked to think about how they felt the previous week before answering the questions. The answers for both the PAQLQ and the PACQLQ were given on a 7-point scale where a score of 1 represents severe impairment of QoL (not bothered at all) and a score of 7 represents no impairment of QoL (extremely bothered). For the first 3 questions of the PAQLQ, the child had the option to choose an activity where he/she experienced the most

impairment. The PA(C)QLQ has been validated in children with asthma or their caregivers [19]. PAQLQ and PACQLQ were assessed in children ≥ 6 years old and their caregivers, respectively.

For the PA(C)QLQ, assessed end-points were the change from baseline for the overall score and the 3 domains: emotional function score, activity limitation score at t=3 months and t=6 months. Furthermore, change from baseline to t=3 months and t=6 months in the symptoms domain score was assessed for the PACQLQ.

For medication adherence, the MARS-5 questionnaire was used. It consisted of 5 questions where answers were given on a 5-point scale. The MARS-5 questionnaire was filled in by the patient with the help of their parents regarding self-reported adherence. A score of 1 represented 'always' and a score of 5 represented 'never'. The total score thus ranges between 5 and 25.

Data-management

The data of this study was stored in an electronic data management platform system, Castor. After the inclusion of the data from all the patients, the data was extracted from Castor and stored as raw data in an SPSS file in the G-schijf at CDW (Central Digital Workplace). Data cleaning and analyses were done in SPSS, all stored in the G-schijf at CDW.

The study was monitored by an independent monitor. The monitor's qualifications including the received GCP training were documented. The monitor verified whether the rights and well-being of human subjects were protected; whether the reported trial data were accurate, complete, and verifiable from source documents and whether the conduct of the trial complied with the currently approved protocol.

Ethics

The protocol was previously approved by the Amsterdam UMC medical ethics committee (NL63849.018.17).

Covariates

The primary endpoint was adjusted for 5 covariates: age, gender, ethnicity, academic/non-academic hospital and season of inclusion. Data describing these covariates were obtained during the first study visit (baseline). The statistical analysis was adjusted for baseline PA(C)QLQ and for the 5 covariates plus baseline PA(C)QLQ.

The reasoning behind adjusting for the covariates and baseline PA(C)QLQ is to gain narrower confidence intervals and greater power to detect an effect. The target population has variability and therefore detecting a treatment effect may be more difficult. When prespecified factors/covariates are incorporated and measured, detecting an effect is done more accurately. [20]

Statistical analysis

Information regarding the demographics of the study population was calculated. Either the median (with IQR) or the mean (with SD) was calculated regarding the continuous variables. This decision was based on whether the variable was normally distributed or not (see supplementary table S3).

A clinically meaningful response was defined as an improvement from baseline of ≥0,5 points (MCID) of the PA(C)QLQ. This applies to both the overall score and the individual domain scores. The minimal clinically important difference is a patient-derived score that reflects a change in a clinical intervention that is meaningful for the patient. This means that this value indicates the smallest change in score in which the patient perceives an improvement.

In total, the PAQLQ consisted of 23 questions including 3 domains: activity limitation (5 questions), emotional function (8 questions) and symptoms (10 questions). The PACQLQ consisted of 13 questions including 2 domains: activity limitation (4 questions) and emotional function (9 questions) [21][22].

To calculate the overall mean score and the domain scores, no transformation of units was necessary. The questions in both the PAQLQ and the PACQLQ were equally weighted. The individual mean domain scores and the overall scores were only calculated if they met the requirements according to the rules of missing data. All total number of responses were added together and divided by the number of responses. The individual domain scores were analyzed in the same way: the responses were added together for each of the domains and divided by the number of responses. This implies that a domain with 5 questions and a domain with 9 questions will both have a mean domain score between 1 and 7 [21][22].

The requirements for missing data and calculating the mean scores were different for the PAQLQ and the PACQLQ. For the PAQLQ the missing data was taken into account as follows: for the overall score, it was never allowed to have more than 2 missing responses. For the symptom and emotional domain, only one missing value per domain was permitted. For the activity limitation domain, no missing responses were accepted. It was different for the PACQLQ because the number of questions in the questionnaire differed from the number of questions in the PAQLQ. For the PACQLQ, it was never accepted to have more than 1 missing response/answer for the overall score and no missing responses were allowed for each of the domains [21][22].

Changes from the baseline of the overall score and the domain scores PA(C)QLQ were analyzed using a mixed effect model with repeated measures (MMRM) approach. Data analysis using SPSS was carried out. The analysis included the covariates as fixed effects and the intercept of every patient at a certain time point within the study period as a random effect. It is a model where there is a separate mean parameter for each time point in each arm (t=0, t=3, and t=6 months). The value of the regression coefficient of each of the regression lines was averaged across the visits. In the end, every patient had an averaged regression and patients between the intervention arm and the control arm were compared. [23]

A score of 23 was the cut-off point for the MARS-5 questionnaire to determine when a patient is non-adherent to their medication. There is no gold standard for the chosen cut-off value. Based on Koster et al [24] a cut-off point of 23 was chosen. Patients with scores of 23 or higher were considered to be highly adherent. The total score was only calculated when the answers to all 5 questions were given. To investigate whether the adherence significantly differs between the two arms, a one-way ANOVA test was performed.

Data were analyzed using an intention-to-treat approach (ITT). An ITT approach is an approach in which patients are analyzed as randomized. This means that all patients who are randomized, are also included in the analysis according to the arm they were initially assigned to [25].

Results

Study patients

In total 192 patients were assessed for eligibility. 102 patients were randomized to either the control arm (n=52) or the intervention arm (n=50) (figure 3). Baseline demographics and patient characteristics were broadly similar across the control arm and the intervention arm (table 1). In both arms, baseline PA(C)QLQ scores were comparable. However, there are important differences between the arms with a greater proportion of patients included in summer in the control arm (18% versus 30.7%) and a greater proportion of patients included in the winter in the intervention arm (42% versus 34.6%). Furthermore, comedication used at baseline was mainly for allergic rhinitis or allergic conjunctivitis.

3 patients in the intervention arm (6.0%) and 5 patients in the control arm (9.6%) had their study period during the lockdown of schools (supplementary table S11). Respectively 23 and 23 patients in the intervention arm and the control arm had their entire study period before COVID-19. Respectively 25 and 29 patients in the intervention and the control arm had a part or their entire study period during COVID-19. Randomization dates of 2 patients were missing (supplementary table S12).

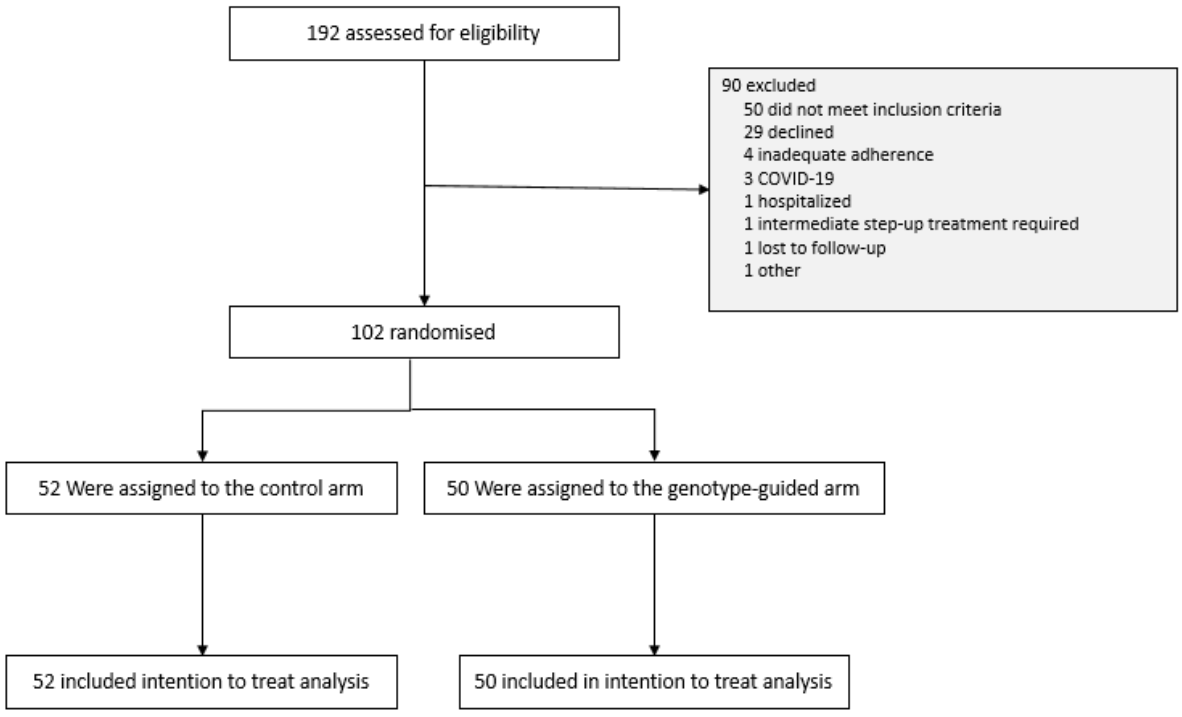

Figure 3: Trial profile

Table 1: Demographic and medical characteristics at t=0 (baseline) ADRB2-genotype guided group vs control group

	Intervention arm	Control arm
Patients	50 (49.0)	52 (51.0)
Age years	10 [8-12]	10 [8-13]
Age group		
< 12 years	35 (34.3)	32 (31.4)
≥ 12 years	15 (14.7)	20 (19.6)
Gender		
Female	20 (60)	28 (53.8)
Male	30 (40)	24 (46.2)
Genotype		
Gly16Gly	23 (46)	19 (36.5)
Arg16Arg	5 (10)	11 (21.2)
Arg16Gly	22 (44)	22 (42.3)
Race/ethnicity		
Dutch	40 (80)	38 (73.1)
Turkish	0 (0.0)	0 (0.0)
Moroccan	5 (10)	1 (1.9)
Surinamese	0 (0.0)	3 (5.8)
Antillean	0 (0.0)	0 (0.0)
Other	5 (10)	10 (19.2)
BMI (kg/m2)*	0.0605±1.17	-0.0582±0.81
Baseline PA(C)QLQ		
Overall score	5.15 ± 1.24	5.33 ± 0.91
Activity score	4.94 ± 1.54	5.01 ± 1.31
Emotion score	5.56 ± 1.17	5.66 ± 0.85
Baseline PAQLQ		
Symptom score	4.15 ± 1.31	4.87 ± 1.09
Baseline MARS questionnaire	23.00 [21.00-25.00]	23.00 [21.00-24.00]
Academic/non-academic hospital		
Academic hospital	8 (16)	10 (19.2)
Non-academic hospital	42 (84)	42 (80.8)
Season of inclusion		
Spring	8 (16)	6 (11.5)
Summer	9 (18)	16 (30.7)
Autumn	12 (24)	12 (23.1)
Winter	21 (42)	18 (34.6)
Baseline comedication		
Allergic rhinitis **	31 patients	33 patients
Allergic conjunctivitis ***	0 patients	2 patients
Eczema/psoriasis ******	2 patients	2 patients

Data are presented as n, mean±SD, median [rangelower, rangeupper]or n(%).
*BMI scores presented as z-score±SD, ** dymista, loratadine, desloratadine, fexofenadine, flixonase, avamys, mometasone nasal spray or levocetirizine *** zaditen eyedrops or levocobastine eyedrops **** Lanette crème or triamcinolone crème or elocon

Quality of life (PA(C)QLQ)

Prescription of a LABA or doubling the dosage of ICS based on ADRB2-genotype did not result in a significant improvement in mean overall PA(C)QLQ score compared to the control arm. The overall difference in overall PA(C)QLQ score was -0.114 (95% CI -0.422-0.194; p=0.463) adjusted for baseline PA(C)QLQ and -0.079 (95% CI -0.384-0.226; p=0.608) for adjusted for baseline PA(C)QLQ and the covariates. At 3 and 6 months the difference in change in mean overall PA(C)QLQ score adjusted for baseline PA(C)QLQ and covariates were respectively -0.059 (-0.408-0.290; p=0.737) and -0.092 (95% CI -0.446-0.263; p=0.610) (table 2).

Table 2: Difference in change in quality of life (PA(C)QLQ) overall scores after 3 and 6 months

	Adjusted for baseline[#] Difference (95%CI)	**p-value**	**Adjusted* Difference (95%CI)**	**p-value**
Overall difference	-0.114 (-0.422-0.194)	0.463	-0.079 (-0.384-0.226)	0.608
At 3 months	-0.091 (-0.444-0.261)	0.610	-0.059 (-0.408-0.290)	0.737
At 6 months	-0.130 (-0.488-0.228)	0.474	-0.092 (-0.446-0.263)	0.610

95%CI: 95% confidence interval, # adjusted for baseline (PA(C)QLQ), * adjusted for age, sex, ethnicity, type of hospital, season and baseline (PA(C)QLQ).

There was no difference in improvement in QoL in either the 3 domain scores at 3 months, 6 months, or the overall difference according to tables 3, 4 and 5. When adjusting for covariates and baseline PA(C)QLQ, the activity score after 6 months showed a difference of -0.074 (-0.523-0.377; p=0.746) and the emotion score showed a difference of -0.094 (-0.411-0.224; p=0.561). The symptom score, which was only answered by children over 12 years old (PAQLQ), showed a change of 0.175 (-0.752-1.102; p=0.703) after 6 months when adjusted for covariates and baseline PA(C)QLQ.

The mean scores of QoL (overall score and domain scores) were plotted in the supplementary figures S1-S4. The slopes of the intervention arm show a steeper slope compared to the control arm. For a complete description of all the mean scores (domains and overall score) see supplementary table S7.

Table 3: Difference in change in quality of life (PA(C)QLQ) activity scores after 3 and 6 months

	Adjusted for baseline[#] Difference (95%CI)	**p-value**	**Adjusted* Difference (95%CI)**	**p-value**
Overall difference	-0.013 (-0.435-0.409)	0.952	0.017 (-0.387-0.421)	0.933
At 3 months	0.079 (-0.381-0.540)	0.734	0.109 (-0.334-0.552)	0.627
At 6 months	-0.102 (-0.570-0.366)	0.668	-0.074 (-0.523-0.377)	0.746

95%CI: 95% confidence interval, # adjusted for baseline (PA(C)QLQ), * adjusted for age, sex, ethnicity, type of hospital, season and baseline (PA(C)QLQ).

Table 4: Difference in change in quality of life (PA(C)QLQ) emotion scores after 3 and 6 months

	Adjusted for baseline[#] Difference (95%CI)	**p-value**	**Adjusted* Difference (95%CI)**	**p-value**
Overall difference	-0.135 (-0.385-0.115)	0.287	-0.123 (-0.379-0.133)	0.341
At 3 months	-0.152 (-0.460-0.156)	0.331	-0.140 (-0.453-0.172)	0.376
At 6 months	-0.105 (-0.418-0.209)	0.511	-0.094 (-0.411-0.224)	0.561

95%CI: 95% confidence interval, # adjusted for baseline (PA(C)QLQ), * adjusted for age, sex, ethnicity, type of hospital, season and baseline (PA(C)QLQ).

Table 5: Difference in change in quality of life (PAQLQ) symptom scores after 3 and 6 months

	Adjusted for baseline# Difference (95%CI)	p-value	Adjusted* Difference (95%CI)	p-value
Overall difference	0.017 (-0.770-0.805)	0.964	0.209 (-0.687-1.104)	0.634
At 3 months	0.077 (-0.766-0.921)	0.854	0.281 (-0.652-1.215)	0.544
At 6 months	-0.014 (-0.853-0.826)	0.974	0.175 (-0.752-1.102)	0.703

95%CI: 95% confidence interval, # adjusted for baseline (PA(C)QLQ), * adjusted for age, sex, ethnicity, type of hospital, season and baseline (PA(C)QLQ).

Every patient filled in at least 2 questionnaires. Of the patients in the control arm respectively 90.6% and 85% (PACQLQ and PAQLQ) filled in all 3 questionnaires (baseline, t=3 and t=6). All patients filled in the questionnaire both for PA(C)QLQ (100%) and PAQLQ (100%) at baseline (supplementary tables S8 and S9).

Medication adherence and switching treatment

10 patients (20.0%) in the intervention arm and 12 patients (23.7%) in the control arm were non-adherent to their medication at t=3 (table 6). Additionally, 13 patients (26.0%) in the intervention arm and 12 patients (23.7%) in the control arm switched treatment at t=3. Among the patients who switched treatment at t=3, respectively 2 patients (4.0%) and 6 patients (11.5%) in the intervention arm and the control arm were also non-adherent at t=3.

Table 6: Overview of non-adherence and switching treatment at t=3.

	Intervention arm	Control arm
Non-adherence* at t=0	15 (30)	16 (30.8)
Non-adherence* at t=3	10 (20.0)	12 (23.7)
Non-adherence* at t=6	11 (22)	16 (30.8)
Switch treatment at t=3	13 (26.0)	12 (23.7)
Non-adherence* + switch treatment at t=3	2 (4.0)	6 (11.5)

*Data are presented as n(%). *≤22 score MARS-5.*

The results of the one-way ANOVA test of the MARS scores show that there is no significant difference between the scores in the intervention arm compared to the control arm (table 7).

Table 7: Results of the total score of self-reported adherence according to the MARS-5 questionnaire at t=0, t=3 and t=6.

	Intervention arm	Control arm	P value
T=0	23 [21-25]	23 [21-24]	0.457
T=3	23 [22-25]	23 [21-24]	0.531
T=6	23 [22-25]	23 [22-24]	0.245

Data are presented as median [rangelower, rangeupper].

Discussion

To our knowledge, this is the first study that investigated children with uncontrolled asthma on step 2 of the GINA guidelines. The objective of this study was to determine if genotype-guided treatment improved the quality of life for children suffering from uncontrolled asthma when compared to standard care. Prescribing a LABA or doubling the dosage of ICS according to the ADRB2-genotype resulted in no difference in improvement in quality of life. Nevertheless, the direction of the results suggests a possible benefit for the genotype-guided arm.

Previous studies have investigated the potential benefit of ADRB2 genotype-directed prescribing in pediatric patients with asthma [14][15]. Yet, these studies are mainly observational and are genotype-stratified studies. In contrast to those studies, we set more specific inclusion and exclusion criteria regarding the severity of asthma symptoms. Only children with uncontrolled asthma symptoms at step 2 of the asthma treatment were included. Additionally, the ICS used at baseline was set in the criteria as well. This prevented including children who used more inhaled corticosteroids at baseline, implying more chance of improvement due to more severe asthma symptoms. Furthermore, we included children between the age of 6-18 years old while the previous studies only included children between the age of 12-18 years old. This allowed us to specify the study population.

This clinical trial highlights a detailed study design and method to investigate genotype-guided prescribing to improve quality of life accurately. A notable strength of this research is the fact that the study was a multicenter, international, double-blind trial. Both academic and non-academic hospitals recruited patients and both the treating physician and the patient were blinded to randomization between the intervention arm and the control arm. In addition, longitudinal data was used which enables us to assess trajectories and individual variability in responses to the treatments. Our study also had qualified staff performing the research visits and the study was monitored by an independent monitor. The questionnaires used were validated for the corresponding age of the patients.

The use of an MMRM analysis used in this research efficiently incorporates all available data. This approach accounts for missing data by modeling each patient's trajectory over time. This is crucial given that the multiple measurements for one individual often correlate and therefore are not independent. Taking this correlation into account, using MMRM reflects the outcome among these repeated measurements. It provides a more accurate estimation of the effect on quality of life and applies to the overall study population.

However, this study is not without its constraints, careful consideration of these limitations is essential for an understanding of the findings. The reliability of this study's conclusions was bound to the statistical power. Using the data of the PUFFIN study, 102 patients were included powered for their primary outcome. As a result of the post-hoc power analysis from this study, a power of 4.3% with a sample size of 102 was found. The lack of a greater sample size may interfere with the possibility of observing an actual significant difference. In this study, it was difficult to include children because the changes in medication following this stepwise approach from step 2 to step 3, are mainly done by the general practitioner. Therefore children were easily missed. Only children visiting the hospital for their

routine appointments could be included if they met the inclusion criteria. It is not possible to state with certainty that there is no association. It is plausible that by increasing the sample size the possibility of observing a significant difference in quality of life.

Although medication adherence was an inclusion criterion for our study, the results of the self-reported adherence of the MARS-5 questionnaire showed that not every patient was adherent to their medication. The one-way ANOVA test showed that there was no difference between the scores of the intervention arm compared to the control arm. There are many ways to measure adherence. In our study adherence was evaluated by the treating physician. However, the results of the self-reported adherence suggest a potential non-adherence to medication among 31 patients in the study population at baseline. Additionally, patients kept reporting non-adherence to their medication throughout their study period. This lack of adherence may have contributed to a different use of the prescribed medication. The results say something about the adherence in the study population and not on an individual level. Moreover, social desirability bias might have influenced the outcome parents report of the child's adherence. Medication adherence might even be different outside of a clinical trial. Patients may experience less pressure to take their medication correctly when they do not participate in a study. Thus, although non-adherence was found and the results of the MARS-5 questionnaire were equally divided between the intervention arm and the control arm, patients might have a different approach toward adherence outside of the study and in their daily lives.

Furthermore, the comparison of a LABA and a double dose of ICS may influence the experience of the patient and therefore also the perceived quality of life. The use of a LABA will immediately result in an effect due to the effect of the β2-agonist (targets airway muscle relaxation) compared to the ICS which decreases inflammation and needs to be taken for a longer time to experience effect. It is plausible that this could have led to a lower motivation to continue to use the ICS and thus non-adherence to the medication. Moreover, 6 patients in de control arm and 2 patients in the intervention arm switched treatment after 3 months due to maintaining uncontrolled asthma and were also non-adherent. Of these patients, respectively, 1 and 3 patients switched from double dosage ICS to single dosage ICS plus LABA. In the end, these limitations are dependent on the effort the patients want to make and may not represent the usage outside of the trial.

Lastly, our study was conducted during the COVID-19 pandemic when schools had lockdowns that may have interfered with the quality of life perceived by the child or the caregiver(s). In total 8 patients had their study period during one or more of the lockdowns of schools in the Netherlands. Staying inside and having less to no social contact with others, may on its own already affect the anxiety and concern of the patient. Therefore, the experience and perception of the quality of life of the patient (and their caregivers) during COVID-19 may have influenced the results of the PA(C)QLQ.

In practice, this implies that the treating physicians could consider genotyping for the ADRB2 gene in children who continue to experience uncontrolled asthma. This might give a better understanding of the underlying mechanism of the uncontrolled asthma symptoms despite taking the prescribed asthma medication. In this decision-making, adherence and proper use of asthma medication should be taken

into account. Especially in children, good instruction is crucial because inhalation of asthma medication is not easily done. Furthermore, this study adds external validity to a previous study, the PACT study, that was performed in England and Scotland. In the future, this research could potentially be extrapolated to other countries in Europe and the potential benefit might apply to other European pediatric populations as well.

In conclusion, the insights of this study contribute to a better understanding of the potential value of genotype-guided treatment on the quality of life in children with uncontrolled asthma. The direction shows that quality of life could improve in children with uncontrolled asthma when genotype-guided prescribing is used. In the future, the results of the PACT study will be harmonized with the PUFFIN study to see whether our results could add to their results.

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Supplemental appendix

Supplemental methods

Full list of in- and exclusion criteria

Exclusion criteria:

- Active smoking;
- Congenital heart disease;
- Serious lung disease other than asthma;
- Omalizumab use;
- ICU admission in the previous year.

Inclusion criteria:

- Between 6-18 years of age;
- Doctor's diagnosis of asthma (ever) based on patient history, FEV1 reversibility ≥12% and/or bronchial hyperresponsiveness;
- Current asthma symptoms (based on ACT ≥12 years) or C-ACT (<12 years) score ≤ 19;
- ICS use ≥ 3 months before inclusion (start dosage ICS, treatment step 2 according to childhood asthma guideline NVK;
- Adequate inhalation technique;
- Good adherence to maintenance asthma treatment determined by the treating physician;
- Understanding of Dutch or German language;
- Internet access at home, willing to fill in an internet questionnaire.
- Both the child and the caregiver(s) need to fill in the informed consent.

Table S1: ICS dosing step 2 for inclusion criterium ICS use ≥3 months [1]

ICS	**Dosage (µg)**
Beclomethasone	2dd 200
Beclomethasone (extra fine)	2dd 100
Budesonide	2dd 200
Fluticasone	2dd 100-125
Ciclesonide	1dd 160

Table S2: Possible treatment regimes when uncontrolled asthma at t=3 months

	Arg16Arg or Arg16Gly	**Gly16Gly**	
Therapy: months 0-3	Double ICS	Double ICS	ICS+LABA
Therapy for months 4-6 if still uncontrolled at 3 months	Normal dosage ICS and LTRA	Normal dosage of ICS and LABA	Double ICS

ICS: Inhaled Corticosteroid. LABA: long-acting β-agonist.

Supplemental results

Table S3: Overview of results Shapiro-Wilk test of Normality

	Shapiro-Wilk test of Normality	
	Intervention arm	**Control arm**
Age	0.060	**0.016**
BMI (kg/m2)*	**0,003****	**0,010****
Baseline MARS questionnaire	**0.001**	**<0.001**
T=3 MARS questionnaire	**<0.001**	**<0.001**
T=6 MARS questionnaire	**<0.001**	**<0.001**
Mean_overallscore_t0	0.583	0.524
Mean_activityscore_t0	0.556	0.776
Mean_emotionscore_t0	0.141	0.094
Mean_symptomscore_t0	0.776	0.703
Mean_overallscore_t3	**0.001**	0.069
Mean_activityscore_t3	0.749	**0.030**
Mean_emotionscore_t3	**<0.001**	0.158
Mean_symptomscore_t3	**<0.001**	0.482
Mean_overallscore_t6	0.430	0.263
Mean_activityscore_t6	0.454	0.116
Mean_emotionscore_t6	0.140	**0.026**
Mean_symptomscore_t6	0.298	0.059

*BMI: Body Mass Index. **Value for demographic table not based on Shapiro-Wilk test of Normality to choose whether mean or median, but Z-score ± SD is used.

If the result of the Shapiro-Wilk test of normality had a significance of <0,05, the null hypothesis is rejected. The null hypothesis states that the variable is normally distributed. If this was the case in either the control arm or the intervention arm, the median and the IQR were used. The median and IQR are then used for BOTH arms AND for every timepoint. This is done to easily compare the scores between each timepoint (either ALL median and IQR or ALL mean and SD in one table).

The z-scores were calculated for the BMI. The z-score was used as a standard score. It shows how many standard deviations the variable deviates from the mean of a group of values. In other words, it specifies the location of a measurement within a group/distribution. The advantage of using a z-score is that you can directly see where observation A lies compared to other observations (and the mean). A negative Z-score represents an observation below the mean of the sample. A positive Z-score represents an observation above the mean [2].

Post-hoc power analysis explanation

Data for this research was obtained from the PUFFIN study. The PUFFIN study aimed at 310 children based on their primary endpoint ACT scores. With a post-hoc power analysis, a calculator should be used that takes time points and interactions into consideration. This is due to the use of a mixed model analysis. Because the outcome of this study only uses 2 data points (the difference between before and after intervention), a simple calculator is used. To determine the power for the primary endpoint PA(C)QLQ, 'Clincalc' is used [3]. The measured mean end scores of PA(C)QLQ were used with an alpha of 0.05.

Table S4: Study parameters and chosen values in power analysis calculator.

Study parameter/criteria	**Chosen value/criteria in 'Clincalc'[3]**
Endpoint	Continuous
Sample study	Two independent sample study
Mean group 1 +/- SD (intervention arm; overall score)	6.25 ± 0.88
Mean group 2 +/- SD (control arm; overall score)	6.21 ± 0.79
Subjects, group 1 (intervention arm)	50
Subjects, group 2 (control arm)	52
Alpha	0.05

➔ The post-hoc power analysis showed a statistical power of **4.3%.**

Table S5: Number of patients using comedication at baseline, categorized.

Category comedication	**Number of patients**
ADHD	1
Allergic rhinitis	64
Allergic conjunctivitis	2
Anaphylaxis/bronchospasms	4
Anemia	1
Asthma	85
Birth control	1
Dry cough	1
Eczema/psoriasis	4

ADHD: Attention Deficit Hyperactivity Disorder.

Table S6: Overview of comedication in each category.

Category comedication	Name	ATC-codes
ADHD	Methylphenidate (Equasym)	N06BA04
Allergic rhinitis	Azelastine/Fluticasone nasal spray (Dymista)	R01AD58
	Loratadine (Claritine)	R06AX13
	Desloratadine (Aerius)	R06AX27
	Fexofenadine	R06AX26
	Fluticasone propionate (Flixonase)	R01AD08
	Fluticason furoate (Avamys)	R01AD12
	Mometason nasal spray	R01AD09
	Levocetirizine (Xyzal)	R06AE09
Allergic conjunctivitis	Zaditen eye drops	S01GX08
	Levocabastine eyedrops	R01AC02
Anaphylaxis, bronchospasms	EpiPen (adrenaline)	C01CA24
Anemia	Ferrofumarate	B03AA02
Asthma	Salbutamol (Ventolin) Flixotide	R03AC02 R03BA05
Birth control	Ethinylestradiol/levonorgestrel	G03AA07
Dry cough	Noscapine	R05DA07
Eczema/psoriasis	Lanette creme	D02AX
	Triamcinolone creme	D07AB09
	Elocon (mometasone)	D07AC13

ADHD: Attention Deficit Hyperactivity Disorder. ATC- code: Anatomical Therapeutic Chemical – code.

Table S7: Overall scores and domain scores of PA(C)QLQ at t=0, t=3 and t=6.

	Intervention arm				**Control arm**			
	Activity	Emotion	Symptom	Overall	Activity	Emotion	Symptom	Overall
T=0	5.25 [4.00-6.33]	5.78 [4.67-6.50]	4.00 [2.90-5.50]	5.31 [4.48-6.00]	5.20 [3.80-6.00]	5.75 [5.22-6.33]	5.00 [4.05-5.65]	5.48 [4.77-6.00]
T=3	6.25 [4.75-7.00]	6.67 [5.78-6.94]	5.55 [4.40-5.80]	6.16 [5.54-6.92]	6.45 [4.75-7.00]	6.44 [5.56-6.88]	5.70 [3.90-6.30]	6.31 [5.57-6.69]
T=6	6.75 [5.60-7.00]	6.59 [6.17-7.00]	6.00 [5.00-6.30]	6.46 [5.87-7.00]	6.38 [5.25-7.00]	6.67 [5.89-7.00]	6.20 [5.60-6.80]	6.46 [5.85-6.85]

Table S8: Overview of number of patients who filled in the PA(C)QLQ questionnaires on t=0, t=3 and t=6.

	Control arm		**Intervention arm**	
	PACQLQ	PAQLQ	PACQLQ	PAQLQ
Filled in at baseline	32 (100.0)	20 (100.0)	35 (100.0)	15 (100.0)
Filled in at t=3	32 (100.0)	19 (95.0)	34 (97.1)	14 (93.3)
Filled in at t=6	29 (90.7)	18 (90.0)	33 (94.3)	15 (100.0)
Patients total in arm	32	20	35	15

PA(C)QLQ: Pediatric Asthma (Caregiver) Quality of Life Questionnaire.

Table S9: Overview of number of patients who filled in more than 2 and 3 PA(C)QLQ questionnaires.

	Control arm		**Intervention arm**	
	PACQLQ	PAQLQ	PACQLQ	PAQLQ
Filled in ≥2 questionnaires	32 (100.0)	20 (100.0)	35 (100.0)	15 (100.0)
Filled in = 3 questionnaires	29 (90.6)	17 (85.0)	32 (91.4)	14 (93.3)
Patients total in arm	32	20	35	15

PA(C)QLQ: Pediatric Asthma (Caregiver) Quality of Life Questionnaire.

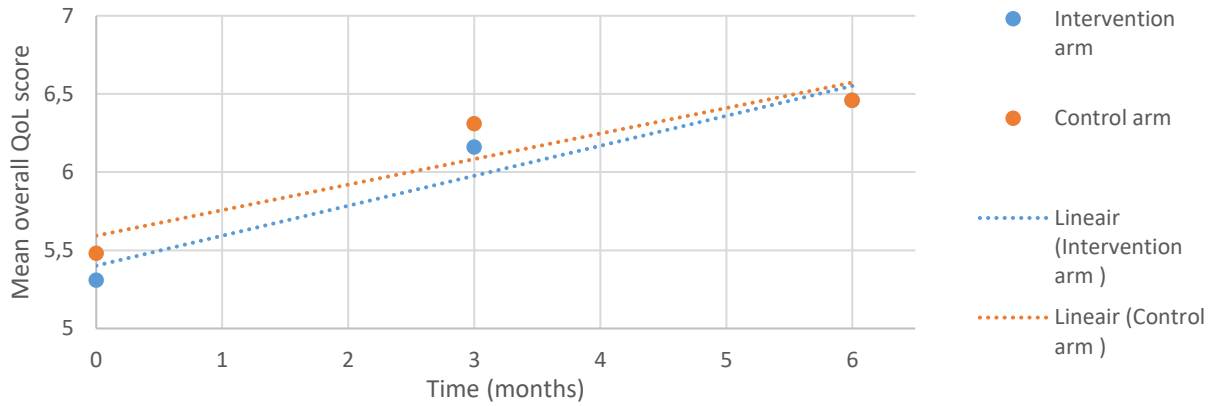

Figure S1: Mean overall score during study period of PA(C)QLQ.

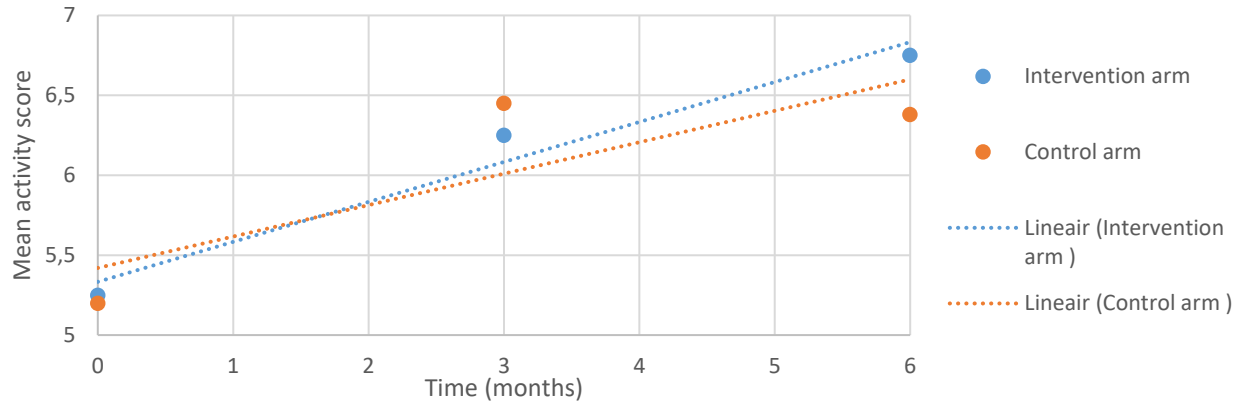

Figure S2: Mean activity score during study period of PA(C)QLQ.

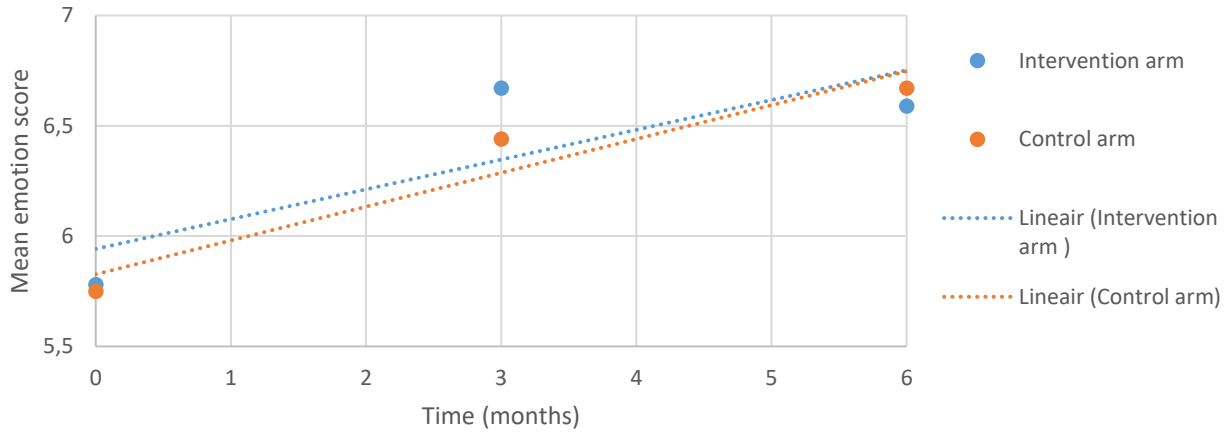

Figure S3: Mean emotion score during study period of PA(C)QLQ.

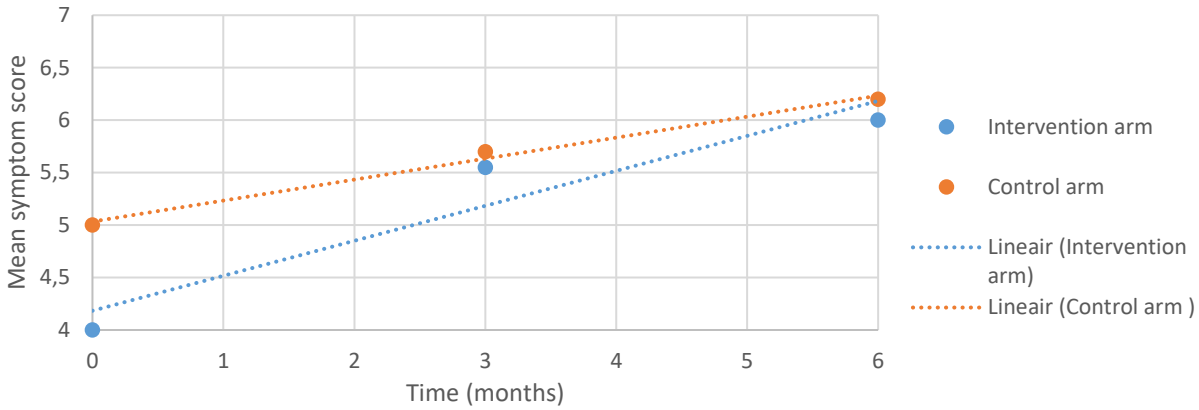

Figure S4: Mean quality of life symptom score of PAQLQ.

Table S10: Overview of patients with non-adherence* at t=0, t=3 and t=6 according to the MARS-5 questionnaire.

	Intervention arm	Control arm
T=0	**15 (30)**	**16 (30.8)**
Missing data# t=0	12 (24)	9 (17.3)
T=3	**10 (20)**	**12 (23.1)**
Missing data# t=3	18 (36)	15 (28.8)
T=6	**11 (22)**	**16 (30.8)**
Missing data# t=6	18 (36)	17 (32.7)

*Data is presented as n(%); *≤22 score MARS-5. #total score is not calculated if not all the 5 questions were answered.*

Table S11: Overview of the number of patients that had their study period during the lockdown of schools in the Netherlands during COVID-19.

	Intervention arm	Control arm
16-Mar-2020 until 08-May-2020*	1 (2.0)	1 (1.9)
14-Dec-2020 until 08-Feb-2021*	1 (2.0)	2 (3.8)
14-Dec-2021 until 10-Jan-2022*	1 (2.0)	2 (3.8)
Total patients	3 (6.0)	5 (9.6)

*Data are presented as n(%).*based on information from RIVM on the lockdown of schools [4]. COVID-19: coronavirus disease 2019.*

Table S12: Overview of the number of patients that had their study period before or (partly) during COVID-19

	Intervention arm	Control arm
Entire study period before COVID-19*	23	23
Study period (partly) during COVID-19*	25	29
Missing date of randomization	2	

**period of COVID-19 based on the start of February 2020 in the Netherlands. Taking into account the study duration of 6 months: patients with a randomization date of August 2019 and earlier were included BEFORE COVID-19.*

Supplemental references

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