Fluticasone Propionate Pharmacogenetics: *CYP3A4*22* Polymorphism and Pediatric Asthma Control

Chris Stockmann, MSc[1,2], **Bernhard Fassl, MD**[1], **Roger Gaedigk, PhD**[3], **Flory Nkoy, MD, MPH, MS**[1], **Derek A. Uchida, MD**[1], **Steven Monson, BS**[1], **Christopher A. Reilly, PhD**[2], **J. Steven Leeder, PharmD, PhD**[3], **Garold S. Yost, PhD**[2], and **Robert M. Ward, MD**[1,2]

[1]Department of Pediatrics, University of Utah School of Medicine

[2]Department of Pharmacology/Toxicology, University of Utah College of Pharmacy

[3]Developmental Pharmacology and Experimental Therapeutics Laboratory, University of Missouri Kansas City

Abstract

Objective—To determine the relationship between allelic variations in genes involved in fluticasone propionate (FP) metabolism and asthma control among children with asthma managed with inhaled FP.

Study design—The relationship between variability in asthma control scores and genetic variation in drug metabolism was assessed by genotyping nine single nucleotide polymorphisms (SNPs) in *CYP3A4, CYP3A5*, and *CYP3A7*. Genotype information was compared with asthma control scores (0 = well-controlled to 15 = poorly-controlled), determined by using a questionnaire modified from the National Heart Lung and Blood Institute Expert Panel 3 guidelines.

Results—Our study cohort was comprised of 734 children with asthma (mean age 8.8 ± 4.3 years), who were predominantly male (61%) and non-Hispanic Whites (53%); 413 children (56%) were receiving inhaled glucocorticoids daily, of which FP was prescribed most frequently (65%). Among the children receiving daily FP, SNPs in the genes *CYP3A5* and *CYP3A7* were not associated with asthma control scores. In contrast, asthma control scores were significantly improved among 20 (7%) children with the *CYP3A4*22* allele (median 3, range 0-6), as compared with the 201 patients without the *CYP3A4*22* allele (median 4, range 0-15) (*P*=0.02). The presence of *CYP3A4*22* was associated with improved asthma control scores by 2.1 points (95% CI: 0.5-3.8).

Conclusions—The presence of *CYP3A4*22*, which is associated with decreased hepatic CYP3A4 expression and activity, was accompanied by improved asthma control among FP treated children. Decreased CYP3A4 activity may improve asthma control with inhaled FP.

Keywords

asthma; fluticasone; inhaled glucocorticoids; corticosteroids; children; pharmacogenetics

---

Corresponding Author: Robert M. Ward, MD University of Utah Health Sciences Center 295 Chipeta Way, Neonatology Salt Lake City, UT 84108 Ph: 801-581-4178 Fx: 801-585-7395.

Inhaled glucocorticoids are the most effective treatment for controlling chronic asthma symptoms and are commonly prescribed to reduce airway inflammation and bronchial hyper-responsiveness.[1, 2, 3] Despite broad use of inhaled glucocorticoids, many patients have poor control of asthma symptoms.[4, 5] These pharmacodynamic differences may be explained, at least in part, by individual variation in pharmacokinetics or pharmacogenetics.

Fluticasone propionate (FP), a trifluorinated synthetic glucocorticoid, is one of the most widely used inhaled glucocorticoids for the treatment of asthma. This likely relates to its high immunosuppressive potency when measured with a whole-blood lymphocyte proliferation assay.[6] The biological activity of FP is terminated by metabolic biotransformation, attributed primarily to cytochrome P450 3A enzymes CYP3A4, CYP3A5, and CYP3A7.[7] CYP3A5 is the predominant CYP3A isoform expressed in human lung; however, the most important hepatic 3A enzyme, CYP3A4, is not usually expressed in human lung.[8] We have shown that *CYP3A4* expression in human lung is repressed by factors that bind to upstream transcriptional regulation sites of the *CYP3A4* gene.[9] CYP3A7 is the predominant form of CYP3A expressed in human fetal liver,[10] and our preliminary studies have shown that it is expressed at low, but detectable levels in lung samples in patients up to 35 years old. These data suggest that CYP3A5 and/or CYP3A7 could be responsible for local biotransformation of FP in the lung due to their higher expression in bronchial mucosa and pulmonary parenchyma.[11] Interindividual variability in the response to FP may be explained, in part, by variation in these CYP3A enzymes that mediate FP's biotransformation *in vivo*. Furthermore, FP is a highly potent mechanism-based inactivator of CYP3A5, a less potent inactivator of CYP3A4, and has no action on CYP3A7.[12]

A single nucleotide polymorphism (SNP) in CYP3A5 (*CYP3A5*3*) was shown recently to reduce the biotransformation of lovastatin, prolonging its half-life and exaggerating its effect on serum cholesterol.[13] We hypothesized that *CYP3A5*3* would be associated with reduced catalytic activity in the lung, thereby increasing local pulmonary concentrations of FP that would improve asthma control in children treated with inhaled FP. We also speculated that functionally reduced activity, caused by SNPs in *CYP3A4* and *CYP3A7*, might be associated with improved asthma control with inhaled FP. Furthermore, we speculate that mechanism-based inhibition of *CYP3A5*, and to a lesser degree *CYP3A4*, by FP may reduce the rate of FP metabolism and improve asthma control.

Methods

Saliva samples were obtained prospectively from children 2-17 years of age with a physician-confirmed diagnosis of asthma. Subjects were randomly recruited as a convenience sample from the emergency department and inpatient wards of Primary Children's Medical Center in Salt Lake City, Utah according to the availability of the study coordinator. Information about chronic medical conditions, concomitant medication use and chief complaint was collected through a combination of structured interviews and medical chart abstraction. Data were de-identified before genotypic analyses.

Patient levels of chronic asthma control were determined by questionnaire, based upon guidelines, modified from the National Heart Lung and Blood Institute Expert Panel Report 3, which avoided variations in the child's ability to cooperate with pulmonary function testing.[3] The definition of chronic asthma control by Juniper et al as the "full range of clinical impairment that patients with asthma may experience as a result of their disease" was utilized in the development of the questionnaire to assess chronic asthma control among children and young adults.[14] Five questions were posed to the child or parent pertaining to the child's level of chronic asthma control and were quantified using a 4-point scale (Appendix; available at www.jpeds.com). The items were equally weighted, and the

subject's asthma control score was calculated as the sum of all five items. The American Thoracic Society (ATS) and the European Respiratory Society recommend the use of numeric measures of asthma control over categorical variables.[15] ATS guidelines support the classification of patients whose asthma control scores fall between poorly-controlled and well-controlled.[16] In this study, asthma control scores were analyzed as a numeric variable that could range from 0 (well-controlled) to 15 (poorly-controlled).

Oragene DNA kits (DNA Genotek Inc, Ottawa, ON, Canada) were used to collect 2 mL of saliva per patient. The DNA was extracted from 400 µL of saliva/Oragene buffer mix with a silica based column preparation (GeneElute Mammalian Genomic DNA Miniprep Kit, Sigma, St Louis, MO, USA) according to the manufacturer's provided protocol. The genomic DNA was eluted from the column with 10 mM Tris-HCl, pH 8.0, and the DNA concentrations were determined by spectrophotometry (NanoDrop, Thermo Scientific, Wilmington, DE, USA). Samples that had concentrations of >1 ng/µL after the column elution were concentrated by lyophilization and resuspended in 1/10 of the original volume.

All genotype analyses were conducted in the Developmental Pharmacology and Experimental Therapeutics Laboratory within the Division of Clinical Pharmacology and Medical Toxicology at Children's Mercy Hospitals and Clinics (Kansas City, MO). Genotype analysis of nine different SNPs in the *CYP3A* gene locus on chromosome 7, encompassing the genes *CYP3A4, CYP3A5,* and *CYP3A7*, were performed (Table I; available at www.jpeds.com).[13, 17-22] The relative location of these SNPs to each other is shown in Figure 1 (available at www.jpeds.com). Eight of the assays are available from Applied Biosystems (Grand Island, NY, USA). The primers and probes for rs2740565 were custom made by Biosearch Technologies, Inc. (Novato, CA, USA; Table I). Approximately 10 ng of genomic DNA was used for each genotyping assay. After denaturing the DNA by heating for 2 min at 95°C, the TaqMan reactions were cycled 50 times (denaturation for 10 seconds at 95°C, annealed/elongated at 60°C for 1 min) on an Applied Biosystems 7900HT Fast Real-Time PCR System instrument. Data clustering analysis was done with the TaqMan Genotyper software (Applied Biosystems).

Only two samples did not amplify even after several repeat attempts in two of the assays. For all assays, known homozygous wild-type, heterozygous, and homozygous variant DNA samples (the latter genotype was not available for *CYP3A4*22*) were included on all the plates as positive controls. In addition, as a quality control for genotype calling we randomly selected 10% from all of the samples for each of the nine TaqMan assays and reanalyzed them under the same conditions. All original calls were confirmed. Genetic testing was blinded to both treatment and asthma control score.

Study data were collected and managed using REDCap® electronic data capture tools, hosted at the University of Utah. REDCap (Research Electronic Data Capture) is a secure, web-based application, designed to support data capture for research studies, providing: 1) a graphical user interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources.

This study was approved by the University of Utah and Primary Children's Medical Center's Institutional Review Boards. Prior to the performance of any study-related procedures, informed parental permission was provided by the legally-authorized representative of all study subjects and assent was obtained when appropriate.

Statistical analyses

Data were summarized as means (± standard deviation) or medians (range). Hardy-Weinberg equilibrium (HWE) and the χ^2 test of HWE were determined for *CYP3A* genetic polymorphisms. For univariable group comparisons, unordered categorical variables were compared, using a χ^2 test or Fisher exact test, when expected cell counts were less than 5. We used the Shapiro-Wilk test for normality and found that the distribution of asthma control scores was highly skewed ($P<0.001$). Due to the skewed nature of our asthma control scores, a non-parametric Fisher-Pitman permutation test for independent samples was used to compare the association between *CYP3A* genetic polymorphisms and asthma control scores. The Fisher-Pitman test, which assumes a continuously scaled variable, is as powerful as the independent groups *t*-test, but without the assumption of a normal distribution.[23] Differences were considered significant at the $P<0.05$ level. The Bonferroni procedure was used to adjust for 3 pairwise group comparisons (3 SNPs) for each CYP3A enzyme.[24] Stata 11.2 (StataCorp LP, College Station, TX, USA) was used to perform all statistical analyses.

Results

Among 734 children with asthma who provided saliva samples, patients were predominantly male (61%) and non-Hispanic Whites (53%). The mean age was 8.8 (± 4.3) years (Table II). Four-hundred and thirteen (56%) children with asthma were receiving inhaled glucocorticoids daily, of which FP was prescribed most frequently (65%). Of the 268 children receiving daily FP, the median asthma control score was 4 (range 0-15). There was no difference in the age, sex, or race / ethnicity of children receiving daily FP when compared with the overall asthma cohort. However, despite no difference in mean height (123.2 ± 24.1 cm vs. 126.2 ± 28.4 cm; $P=0.5$), FP treated patients had a lower mean body weight (31.7 ± 21.2 kg vs. 37.3 ± 21.7 kg; $P=0.003$).

DNA quantification

Saliva was collected from all 734 enrolled subjects for DNA extraction. The DNA yield varied substantially among individuals; the average concentration was 32.2 ng/µL (± 53.5 ng/µL) with a median of 17.4 (>1-821.03) ng/µL. Ninety-five percent of the samples had DNA concentrations which met the Applied Biosystems' recommendations for TaqMan reactions of 1-20 ng of DNA per reaction.[25]

SNP genotyping

FP was the only inhaled glucocorticoid for which the sample size was sufficient to study the distribution of allelic variants among the 9 SNPs in the *CYP3A* gene locus. Nine probe hydrolysis (TaqMan) based genotyping assays were performed upon 734 saliva samples with a success rate of 99.96%. Genotyping results were available for all 268 patients who were treated daily with FP, and *CYP3A* SNPs were compared with their asthma control scores (Table III). Variant allele frequencies ranged from 1% to 33% and differed among individual SNPs. Two SNPs deviated from Hardy-Weinberg Equilibrium; however, we were unable to explain these findings in evaluation of the analytical assays or local referral patterns.

Twenty-eight percent of the patients treated with FP daily exhibited *CYP3A5* genetic variants. No associations were observed between *CYP3A5*3* ($P=0.5$), *CYP3A5*6* ($P=0.9$), and *CYP3A5*1D* ($P=1.0$) alleles and asthma control scores in this patient cohort.

For *CYP3A7*, allelic variants, *CYP3A7*2*, and rs2740565 located downstream of the canonical *CYP3A7* (6 nucleotides 5′ of exon 14 of *CYP3A7.1L*) were screened in this

population of children with asthma requiring FP therapy. Homozygosity for the variants tested was infrequent, occurring in 2.6-3.4% of subjects. Individuals heterozygous for a *CYP3A7* genetic variant accounted for 22.8-23.5% of subjects with asthma. Genotypic status, however, was not significantly associated with asthma control scores among patients screened for any of the *CYP3A7* variants.

For the SNPs in *CYP3A4*, no association was found among patients with *CYP3A4* rs2246709 or *CYP3A4* rs4646437 genetic variants. We also assessed rs4646437 and asthma control by sex, as it has been reported to feature sex-dependent functional changes;[19] however, we found no difference by sex. In contrast, there was a significant difference among patients with a *CYP3A4*22* variant T-allele, who were more likely to have lower asthma control scores, indicating improved control, when compared with those who featured the C/C genotype (C/T mean: 2.9 (± 2.2) vs. C/C mean: 5.0 (± 3.7); *P*=0.02) (Figure 2).

CYP3A4*22

Among the 20 children who featured the *CYP3A4*22* variant allele, average asthma control scores were significantly lower compared with the 247 children with asthma who did not feature the SNP (2.9 ± 2.2 vs. 5.0 ± 3.7; *P*=0.02) (Figure 2). Asthma control scores of 0-6 were recorded for children with a *CYP3A4*22* T-allele, and scores among children with the C/C genotype ranged from 0-15 (Figure 3; available at www.jpeds.com). The presence of the variant T-allele was associated with a decreased average asthma control score by 2.10 points (95% CI: 0.46-3.75) out of 15. No subjects were homozygous for the *CYP3A4*22* T-allele.

Discussion

We report variants within the *CYP3A* locus which may explain a portion of the variability in therapeutic response to inhaled FP among children with asthma. Despite the predominance of CYP3A5 and CYP3A7 in human lung tissue, we could not identify an association between several *CYP3A5* and *CYP3A7* polymorphisms and asthma control. Even though CYP3A4 is a minor constituent of the CYP3A enzymes in the lung,[7] a polymorphism in *CYP3A4* was associated with improved asthma control.

We have shown in this study that improved asthma control was associated with the presence of the *CYP3A4*22* allele, which has been shown to decrease hepatic CYP3A4 mRNA expression and enzyme activity.[17] Reduced pulmonary enzyme activity would likely prolong the presence of active FP within the airway and increase its effectiveness. Reduced hepatic CYP3A4 expression would be expected to result in higher FP concentrations in the systemic circulation, but such an effect would be dependent on the amount of drug that is systemically available following inhalation. Available data in children indicate that the mean steady-state concentrations of FP in the systemic circulation range from 107 to 183 pg/mL, depending on the particular inhalation chamber used.[26] FP is a potent inactivator of CYP3A5, and to a lesser extent CYP3A4, such that drug which was not metabolized in the liver may inhibit its own metabolism.[12] Reduced enzyme activity by *CYP3A4*22* has been associated with concentration-related toxicity by cyclosporine, including an increased risk of delayed graft function and decreased creatinine clearance in kidney transplant patients.[27] Additionally, this SNP has been associated with a decreased dose requirement among kidney transplant patients receiving the immunosuppressive agent tacrolimus, which is primarily a CYP3A5 substrate.[28] However, Elens et al found that the combination of *CYP3A4*22* and *CYP3A5*1* exerted a larger effect on cyclosporine clearance than either SNP individually consistent with the multiple enzymatic pathways involved in metabolic clearance of cyclosporine.[27] In the present study, more patients were well-controlled with an asthma score of zero in the group with *CYP3A4*22*. This finding is in agreement with our

hypothesis that reduced enzymatic activity by *CYP3A4*22* increases airway exposure to FP. It is also consistent with the previous observation that the clinical effectiveness of statins at conventional doses is increased by *CYP3A4*22*.

Although the association is evident, the physiologic basis for the association between improved asthma control with FP and *CYP3A4*22* is uncertain. Other possible explanations exist. An enteral or swallowed dose of FP is cleared by first-pass metabolism in the liver and gastrointestinal tract.[29] In the presence of the *CYP3A4*22* allele hepatic *CYP3A4* expression would be reduced, which could theoretically increase systemic exposure. This might also increase adverse effects associated with inhaled glucocorticoids such as adrenal suppression and cataracts. Alternatively, this association with *CYP3A4*22* may be a consequence of linkage disequilibrium with another SNP, which regulates *CYP3A* expression in the lung. As a result of *CYP3A7´s* close proximity to *CYP3A4* it is a likely candidate; however, linkage disequilibrium with *CYP3A5* is also possible.

CYP3A4*22 is located in intron 6 of *CYP3A4* and has not been reported to be in linkage disequilibrium with any known common polymorphisms.[17] Mounting evidence suggests that genetic variants in both coding and non-coding regions of genomic DNA can have functional implications.[30] Genetic variants can alter mRNA levels and protein levels, thereby resulting in phenotypic changes. Several mechanisms have been proposed as potential ways in which an intronic SNP may be associated with disease in humans. Defects in transcription, alternative splicing of the gene transcript, and alterations of RNA processing are all possible mechanisms by which an intronic polymorphism can lead to dysfunctional proteins in different disease states, including inflammatory processes.[31, 32]

The molecular mechanism underlying the direct functional significance of *CYP3A4*22* is unclear. However, Wang et al reported that *in silico* folding of single-stranded DNA and RNA was altered by variation at the site of *CYP3A4*22*, potentially influencing the binding capacity of key regulatory proteins.[17] Functional assessment of *CYP3A4*22* in pediatric asthma patients is required to more thoroughly understand the basis of its effect upon pediatric asthma control with FP. Further research is needed to quantify the impact of this SNP on CYP3A4 activity and FP biotransformation. Even without these additional studies, the correlation between *CYP3A4*22* and improved asthma control with inhaled FP has the potential to improve successful selection of an inhaled glucocorticoid for the treatment of individuals with severe asthma.

The metabolic pathways leading to biotransformation and inactivation of other inhaled glucocorticoids may be influenced in a manner similar to that of FP. For glucocorticoids metabolized by other CYPs, similar mechanisms involving inactivating SNPs in *CYP3A5* and *CYP3A7* may enhance their effectiveness. For example, beclomethasone dipropionate is primarily metabolized by CYP3A5,[33] whose expression level is reduced in patients carrying the *CYP3A5*3*, leading to a similar increase in effectiveness. However, the number of patients in the current study treated with inhaled glucocorticoids other than FP is too small to permit a meaningful analysis of the correlation between specific inactivating SNPs and asthma control scores.

This study has several limitations. We did not directly measure CYP3A expression or activity; however, previous studies have shown that the *CYP3A4*22* SNP decreases total *CYP3A4* mRNA levels and enzyme activity *in vivo*.[17] Furthermore, our effect estimates have wide confidence intervals, reflecting the small sample size of our study population. To minimize the effects of population stratification between study groups, we have recruited all participants from the same children's hospital. Additionally, we did not measure pulmonary function and do not have an objective, physiologic measure of asthma control for the

children included in this analysis. Although pulmonary function tests (PFTs) are objective measures that can be tracked with time and during various interventions, they may not accurately reflect the impact of asthma on the patient's overall health and level of daily functionality.[34] In addition, standard spirometry measurements require patient cooperation that is seldom possible in patients at the youngest age range of our study. We therefore utilized self-reported scoring measures of lung function based upon NIH criteria to stratify subjects according to their degree of asthma control. For young children the questionnaire was frequently completed by the parent/caregiver rather than the child. A study which compared asthma symptom agreement between children and parents revealed that parental reporting may result in a more complete dataset, but is also subject to underreporting of asthma symptoms.[35] Lastly, we are limited by self-reporting of medication compliance and do not have an objective, pharmacologic measure of drug concentrations such as saliva or blood concentrations. The clinical relevance of this finding requires confirmation with a larger sample and external validation in other populations with asthma. Until other mechanisms are identified, our data suggest that patients with decreased CYP3A4 activity may benefit from improved asthma control with inhaled FP.

Acknowledgments

Appendix

Appendix:

Pediatric asthma control scores were calculated by summing the patient's responses (assessed on a 4-point scale) for all of the above questions. Overall score: 0 (well-controlled) – 15 (poorly-controlled).

Please check the box which most accurately describes your/your child's asthma:

1. How often does your child experience asthma symptoms (i.e., cough, wheeze, chest tightness, etc.)?
   - ☐ (0) Less than or equal to 2 days/week
   - ☐ (1) Greater than 2 days/week but not daily
   - ☐ (2) Daily
   - ☐ (3) Several times throughout each day

2. How often does your child use their rescue inhaler (i.e., albuterol or ventolin)?
   - ☐ (0) Less than or equal to 2 days/week
   - ☐ (1) Greater than 2 days/week but not daily
   - ☐ (2) Daily
   - ☐ (3) Several times throughout each day

3. How often does your child experience nighttime awakenings in a month?

   For kids ages 2-4
   - ☐ (0) Never
   - ☐ (1) 1 – 2 times per month
   - ☐ (2) 3 – 4 times per month
   - ☐ (3) > 4 times per month

   OR

   For kids ages 5-17
   - ☐ (0) ≤ 2 x/month
   - ☐ (1) 3 – 4 times per month
   - ☐ (2) > 1 per week but not nightly
   - ☐ (3) Often nightly

4. How often does asthma interfere with your child's normal activity?
   - ☐ (0) No limitation
   - ☐ (1) Minor limitation
   - ☐ (2) Some limitation
   - ☐ (3) Extremely limited

5. In the past year, how often have you sought medical care for your child's asthma (needed urgent care such as ER, hospital, doctor, or was prescribed oral steroids for exacerbation)?
   - ☐ (0) 0 – 1 times in the last 12 months
   - ☐ (1) 2 times in the last 12 months
   - ☐ (2) 3 times in the last 12 months
   - ☐ (3) More than 3 times in the last 12 months

Abbreviations

FP	fluticasone propionate
SNPs	single nucleotide polymorphisms
CYP	cytochrome P450
PFTs	pulmonary function tests
SD	standard deviation
CI	confidence interval
ATS	American Thoracic Society
NIH	National Institutes of Health

References

[1]. Akinbami LJ, Sullivan SD, Campbell JD, Grundmeier RW, Hartert TV, Lee TA, et al. Asthma outcomes: healthcare utilization and costs. J Allergy Clin Immunol. 2012; 129:S49–64. [PubMed: 22386509]

[2]. Barnes PJ. Efficacy of inhaled corticosteroids in asthma. J Allergy Clin Immunol. 1998; 102:531–8. [PubMed: 9802359]

[3]. Expert Panel Report 3 (EPR-3): Guidelines for the Diagnosis and Management of Asthma-Summary Report 2007. J Allergy Clin Immunol. 2007; 120:S94–138. [PubMed: 17983880]

[4]. Chung KF, Caramori G, Adcock IM. Inhaled corticosteroids as combination therapy with beta-adrenergic agonists in airways disease: present and future. Eur J Clin Pharmacol. 2009; 65:853–71. [PubMed: 19557399]

[5]. Tantisira KG, Lasky-Su J, Harada M, Murphy A, Litonjua AA, Himes BE, et al. Genomewide association between GLCCI1 and response to glucocorticoid therapy in asthma. N Engl J Med. 2011; 365:1173–83. [PubMed: 21991891]

[6]. Mager DE, Moledina N, Jusko WJ. Relative immunosuppressive potency of therapeutic corticosteroids measured by whole blood lymphocyte proliferation. J Pharm Sci. 2003; 92:1521–5. [PubMed: 12820156]

[7]. Pearce RE, Leeder JS, Kearns GL. Biotransformation of fluticasone: in vitro characterization. Drug Metab Dispos. 2006; 34:1035–40. [PubMed: 16565171]

[8]. Anttila S, Hukkanen J, Hakkola J, Stjernvall T, Beaune P, Edwards RJ, et al. Expression and localization of CYP3A4 and CYP3A5 in human lung. Am J Respir Cell Mol Biol. 1997; 16:242–9. [PubMed: 9070608]

[9]. Biggs JS, Wan J, Cutler NS, Hakkola J, Uusimaki P, Raunio H, et al. Transcription factor binding to a putative double E-box motif represses CYP3A4 expression in human lung cells. Molecular pharmacology. 2007; 72:514–25. [PubMed: 17548528]

[10]. Leeder JS, Gaedigk R, Marcucci KA, Gaedigk A, Vyhlidal CA, Schindel BP, et al. Variability of CYP3A7 expression in human fetal liver. The Journal of pharmacology and experimental therapeutics. 2005; 314:626–35. [PubMed: 15845858]

[11]. Leclerc J, Tournel G, Courcot-Ngoubo Ngangue E, Pottier N, Lafitte JJ, Jaillard S, et al. Profiling gene expression of whole cytochrome P450 superfamily in human bronchial and peripheral lung tissues: Differential expression in non-small cell lung cancers. Biochimie. 2010; 92:292–306. [PubMed: 20034539]

[12]. Murai T, Reilly CA, Ward RM, Yost GS. The inhaled glucocorticoid fluticasone propionate efficiently inactivates cytochrome P450 3A5, a predominant lung P450 enzyme. Chemical research in toxicology. 2010; 23:1356–64. [PubMed: 20707410]

[13]. Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, et al. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. Nature genetics. 2001; 27:383–91. [PubMed: 11279519]

[14]. Juniper EF, Guyatt GH, Cox FM, Ferrie PJ, King DR. Development and validation of the Mini Asthma Quality of Life Questionnaire. Eur Respir J. 1999; 14:32–8. [PubMed: 10489826]

[15]. Reddel HK, Taylor DR, Bateman ED, Boulet LP, Boushey HA, Busse WW, et al. An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. Am J Respir Crit Care Med. 2009; 180:59–99. [PubMed: 19535666]

[16]. Taylor DR, Bateman ED, Boulet LP, Boushey HA, Busse WW, Casale TB, et al. A new perspective on concepts of asthma severity and control. Eur Respir J. 2008; 32:545–54. [PubMed: 18757695]

[17]. Wang D, Guo Y, Wrighton SA, Cooke GE, Sadee W. Intronic polymorphism in CYP3A4 affects hepatic expression and response to statin drugs. Pharmacogenomics J. 2010

[18]. Bhatnagar V, Garcia EP, O'Connor DT, Brophy VH, Alcaraz J, Richard E, et al. CYP3A4 and CYP3A5 polymorphisms and blood pressure response to amlodipine among African-American men and women with early hypertensive renal disease. American journal of nephrology. 2010; 31:95–103. [PubMed: 19907160]

[19]. Schirmer M, Rosenberger A, Klein K, Kulle B, Toliat MR, Nurnberg P, et al. Sex-dependent genetic markers of CYP3A4 expression and activity in human liver microsomes. Pharmacogenomics. 2007; 8:443–53. [PubMed: 17465708]

[20]. Hustert E, Haberl M, Burk O, Wolbold R, He YQ, Klein K, et al. The genetic determinants of the CYP3A5 polymorphism. Pharmacogenetics. 2001; 11:773–9. [PubMed: 11740341]

[21]. Quaranta S, Chevalier D, Allorge D, Lo-Guidice JM, Migot-Nabias F, Kenani A, et al. Ethnic differences in the distribution of CYP3A5 gene polymorphisms. Xenobiotica; the fate of foreign compounds in biological systems. 2006; 36:1191–200.

[22]. Rodriguez-Antona C, Axelson M, Otter C, Rane A, Ingelman-Sundberg M. A novel polymorphic cytochrome P450 formed by splicing of CYP3A7 and the pseudogene CYP3AP1. The Journal of biological chemistry. 2005; 280:28324–31. [PubMed: 15937338]

[23]. Siegel, S.; Castellan, NJ, Jr.. Nonparametric Statistics for the Behavioral Sciences. 2nd ed. McGraw-Hill; New York: 1988.

[24]. Fisher, RA. The design and analysis of experiments. Oliver & Boyd; Edinburgh and London: 1935.

[25]. TaqMan SNP. Genotyping Assays -- Protocol. Part Number 4332856 Rev. B. Applied Biosystems. Jun.2004

[26]. Khan Y, Tang Y, Hochhaus G, Shuster JJ, Spencer T, Chesrown S, et al. Lung bioavailability of hydrofluoroalkane fluticasone in young children when delivered by an antistatic chamber/mask. J Pediatr. 2006; 149:793–7. [PubMed: 17137894]

[27]. Elens L, Bouamar R, Hesselink DA, Haufroid V, van Gelder T, van Schaik RH. The new CYP3A4 intron 6 C>T polymorphism (CYP3A4*22) is associated with an increased risk of delayed graft function and worse renal function in cyclosporine-treated kidney transplant patients. Pharmacogenet Genomics. 2012; 22:373–80. [PubMed: 22388796]

[28]. Elens L, Bouamar R, Hesselink DA, Haufroid V, van der Heiden IP, van Gelder T, et al. A new functional CYP3A4 intron 6 polymorphism significantly affects tacrolimus pharmacokinetics in kidney transplant recipients. Clin Chem. 2011; 57:1574–83. [PubMed: 21903774]

[29]. Harding SM. The human pharmacology of fluticasone propionate. Respiratory medicine. 1990; 84(Suppl A):25–9. [PubMed: 2287792]

[30]. Cooper DN. Functional intronic polymorphisms: Buried treasure awaiting discovery within our genes. Hum Genomics. 2010; 4:284–8. [PubMed: 20650817]

[31]. Do TN, Ucisik-Akkaya E, Davis CF, Morrison BA, Dorak MT. An intronic polymorphism of IRF4 gene influences gene transcription in vitro and shows a risk association with childhood acute lymphoblastic leukemia in males. Biochim Biophys Acta. 2010; 1802:292–300. [PubMed: 19897031]

[32]. Kwok JB, Hallupp M, Loy CT, Chan DK, Woo J, Mellick GD, et al. GSK3B polymorphisms alter transcription and splicing in Parkinson's disease. Ann Neurol. 2005; 58:829–39. [PubMed: 16315267]

[33]. Somers GI, Lindsay N, Lowdon BM, Jones AE, Freathy C, Ho S, et al. A comparison of the expression and metabolizing activities of phase I and II enzymes in freshly isolated human lung parenchymal cells and cryopreserved human hepatocytes. Drug Metab Dispos. 2007; 35:1797–805. [PubMed: 17627976]

[34]. Spahn JD, Chipps BE. Office-based objective measures in childhood asthma. J Pediatr. 2006; 148:11–5. [PubMed: 16423591]

[35]. Magzamen S, Mortimer KM, Davis A, Tager IB. School-based asthma surveillance: a comparison of student and parental report. Pediatr Allergy Immunol. 2005; 16:669–78. [PubMed: 16343089]
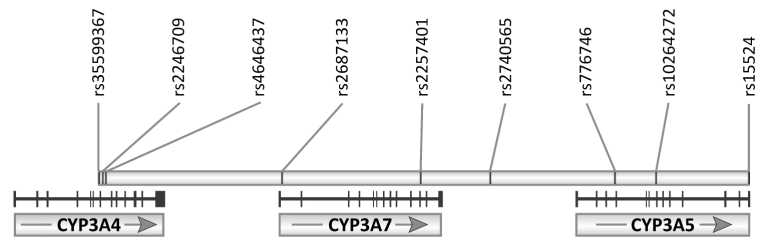
Figure 1.
CYP3A gene locus on chromosome 7. The positions of the genotyped SNPs are indicated relative to the genes *CYP3A4, CYP3A5*, and *CYP3A7*. Vertical bars identify the individual exons, and arrows indicate the direction of transcription..
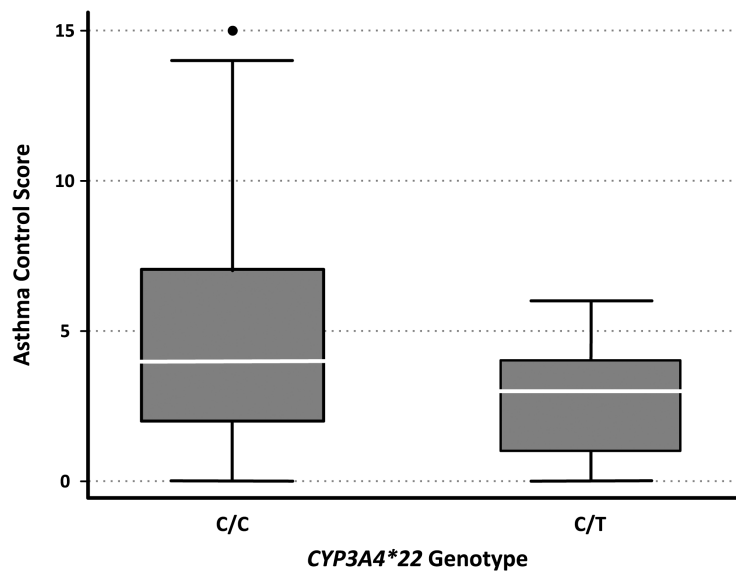
Figure 2.
The box plot depicts pediatric asthma control scores among patients receiving daily fluticasone, stratified by *CYP3A4*22* genotype. Boxes indicate the median and interquartile ranges. Vertical lines above and below boxes indicate the maximum and minimum values. Asthma control scores are scaled from 0 (well-controlled) to 15 (poorly-controlled). The variant T allele was significantly associated with lower asthma control scores, indicating improved asthma control (P=0.02).
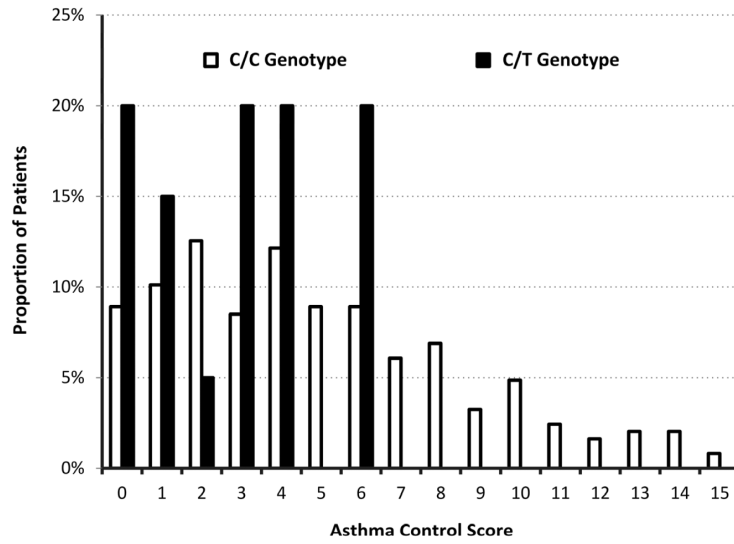
Figure 3.
Asthma control scores stratified by *CYP3A4*22* genotype. Children with asthma featured wild-type (C/C) and variant (C/T) alleles. Scores are ranked from 0 (well-controlled) to 15 (poorly-controlled).

Table 1

Candidate genes for genetic analysis of pediatric asthma control with inhaled fluticasone propionate

SNP	Applied Biosystems Assay #	Reference SNP (rs #)
CYP3A4*22	C__59013445_10	rs35599367
Reported effect: carriers of the variant T allele had lower hepatic CYP3A4 mRNA level and enzyme activity when compared to those with the wild-type C allele.[17]		
CYP3A4 int 7	C___1845287_10	rs2246709
Reported effect: carriers of the variant T allele have improved blood pressure in response to amlodipine treatment among high-risk African Americans.[18]		
CYP3A4 int 7	C__32306227_10	rs4646437
Reported effect: female carriers of the variant T allele had higher CYP3A4 expression and activity than male carriers and even higher expression and activity than males with the wild-type C allele.[19]		
CYP3A5*3	C__26201809_30	rs776746
Reported effect: intronic polymorphism leads to retention of exon 3B, which harbors a premature stop codon.[13,20]		
CYP3A5*6	C__30203950_10	rs10264272
Reported effect: causes the skipping of exon 7 and results in a non-functional protein.[13]		
CYP3A5*1D	C__8303531_40	rs15524
Reported effect: located in the 3′ UTR, 14 nucleotides downstream of a stop codon. Effects upon activity remain unknown.[21]		
CYP3A7	C__26201872_20	rs2687133
No published reports.		
CYP3A7*2	C__25474551_10	rs2257401
Reported effect: an amino acid change (Thr>Arg) in exon 11 results in a more active form of CYP3A7.[22]		
CYP3A7 6 nt 5′ of ex 14	custom	rs2740565
Reported effect: variant A allele results in an alternatively spliced product which is translated into a functional protein which may exhibit different stability and catalytic activity.[22]		
rs2740565 custom assay:		
Forward Primer:	5′ CCCCCATATCTATAAAGTCACAATCC	
Reverse Primer:	5′ TGAATGGGCTCCATATCTACAAAC	
Variant Probe:	5′ TGAGACCTGATTTCTGATT (BHQ plus, FAM)	
Wild-Type Probe:	5′ TGAGACCTGATTTCTGTTT (BHQ plus, TET)	

Table 2

Population characteristics of asthmatic children and young adults who underwent genotyping for CYP3A polymorphisms

Variables	Number (%)
Total no. of subjects	734
Subjects receiving daily inhaled glucocorticoids	413 (56.3)
Fluticasone propionate	268 (36.5)
Flunisolide	10 (1.4)
Triamcinolone acetonide	2 (0.3)
Beclomethasone dipropionate	31 (4.2)
Budesonide	56 (7.6)
Ciclesonide	2 (0.3)
Mometasone	7 (1.0)
Fluticasone propionate / salmeterol xinafoate	82 (11.2)
Budesonide / formoterol	11 (1.5)
Age, yrs	8.8 ± 4.3
Sex	
Male	443 (60.5)
Female	289 (39.5)
Race	
American Indian / Alaskan Native	9 (1.2)
Asian	6 (0.8)
Black	24 (3.3)
Native Hawaiian / Pacific Islander	15 (2.0)
White	554 (75.5)
Unknown / Not reported	122 (16.6)
Ethnicity	
Hispanic	102 (14.0)
Non-Hispanic	211 (28.9)
Unknown / Not reported	418 (57.2)
Weight, kg	35.1 ± 21.6
Height, cm	124.9 ±.26.6
Asthma control score	
Subjects receiving daily fluticasone	4.8 ± 3.6
Subjects receiving other inhaled glucocorticoids	4.9 ± 3.6

NOTE: Values are presented as mean + SD where applicable.

Table 3

Association of *CYP3A* genetic polymorphisms and asthma control scores among children receiving daily inhaled fluticasone propionate

Polymorphism	Reference SNP (rs #)	Test for Hardy Weinberg Equilibrium (*P*-value)	Variant Allele Frequency	Association with Asthma Control Scores (*P*-value) [⊗]
CYP3A4				
CYP3A4*22	rs35599367	0.29	0.04	0.02
CYP3A4 int 7	rs2246709	0.12	0.33	0.42
CYP3A4 int 7	rs4646437	0.03	0.17	0.54
CYP3A5				
CYP3A5*3	rs776746	0.33	0.15	0.51
CYP3A5*6	rs10264272	0.80	0.01	0.86
CYP3A5*1D	rs15524	0.30	0.15	0.96
CYP3A7				
CYP3A7	rs2687133	0.13	0.14	0.55
CYP3A7*2	rs2257401	0.04	0.15	0.50
CYP3A7 6 nt 5′ of ex 14	rs2740565	0.18	0.16	0.69

[⊗]Bonferroni adjustment for multiple comparisons.