HSD3B1 genotype identifies glucocorticoid responsiveness in severe asthma

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Asthma resistance to glucocorticoid treatment is a major health problem with unclear etiology. Glucocorticoids inhibit adrenal androgen production. However, androgens have potential benefits in asthma. HSD3B1 encodes for 3β-hydroxysteroid dehydrogenase-1 (3β-HSD1), which catalyzes peripheral conversion from adrenal dehydroepiandrosterone (DHEA) to potent androgens and has a germine missense-encoding polymorphism. The adrenal restrictive HSD3B1(1245A) allele limits conversion, whereas the adrenal permissive HSD3B1(1245C) allele increases DHEA metabolism to potent androgens. In the Severe Asthma Research Program (SARP) III cohort, we determined the association between DHEA-sulfate and percentage predicted forced expiratory volume in 1 s (FEV1-PP) in healthy cohorts (10, 11) and in asthma (12). Increasing circulating androgens and cortisol is a known consequence of systemic GC treatment. Substantial evidence suggests that stimulation of the androgen receptor (AR) expressed in the lung could be beneficial in asthma. Androgens inhibit human airway smooth muscle and fibroblast proliferation (4–6), promote airway smooth muscle relaxation (7), and inhibit both Th2 and Th1 inflammation in animal models of asthma (8, 9). Androgens are associated with better lung function in large healthy cohorts (10, 11) and in asthma (12). Increasing circulating androgens in response to glucocorticoids is a major clinical problem, and the underlying mechanisms are unknown. It is known that glucocorticoid use can suppress adrenal androgen production. In population studies, animal models, and cell culture experiments, androgens are associated with several benefits in asthma, but neither androgen use in glucocorticoid-resistant asthma nor the genetic determinants of androgen responsiveness have been studied in humans. A missense-encoding variant in HSD3B1 is known to regulate conversion from adrenal precursors to potent androgens and clinical outcomes in prostate cancer. This is the first genetic evidence to our knowledge that implicates an androgen synthesis variant in resistance to glucocorticoids for asthma or any other inflammatory disease. Furthermore, this study demonstrates an adverse consequence of adrenal androgen suppression with glucocorticoid therapy.

Suppression of endogenous adrenal androgens and cortisol is a known consequence of systemic GC treatment. Substantial evidence suggests that stimulation of the androgen receptor (AR) expressed in the lung could be beneficial in asthma. Androgens inhibit human airway smooth muscle and fibroblast proliferation (4–6), promote airway smooth muscle relaxation (7), and inhibit both Th2 and Th1 inflammation in animal models of asthma (8, 9). Androgens are associated with better lung function in large healthy cohorts (10, 11) and in asthma (12). Increasing circulating androgens in response to glucocorticoids is a major clinical problem, and the underlying mechanisms are unknown. It is known that glucocorticoid use can suppress adrenal androgen production. In population studies, animal models, and cell culture experiments, androgens are associated with several benefits in asthma, but neither androgen use in glucocorticoid-resistant asthma nor the genetic determinants of androgen responsiveness have been studied in humans. A missense-encoding variant in HSD3B1 is known to regulate conversion from adrenal precursors to potent androgens and clinical outcomes in prostate cancer. This is the first genetic evidence to our knowledge that implicates an androgen synthesis variant in resistance to glucocorticoids for asthma or any other inflammatory disease. Furthermore, this study demonstrates an adverse consequence of adrenal androgen suppression with glucocorticoid therapy.

Significance

Although resistance to glucocorticoids is a major clinical problem, the underlying mechanisms are unknown. It is known that glucocorticoid use can suppress adrenal androgen production. In population studies, animal models, and cell culture experiments, androgens are associated with several benefits in asthma, but neither androgen use in glucocorticoid-resistant asthma nor the genetic determinants of androgen responsiveness have been studied in humans. A missense-encoding variant in HSD3B1 is known to regulate conversion from adrenal precursors to potent androgens and clinical outcomes in prostate cancer. This is the first genetic evidence to our knowledge that implicates an androgen synthesis variant in resistance to glucocorticoids for asthma or any other inflammatory disease. Furthermore, this study demonstrates an adverse consequence of adrenal androgen suppression with glucocorticoid therapy.

Author contributions: J.Z., B.G., M.D.D., E.R.B., D. Myers, S.C., W.W.B., W.J.C., V.O., G.A.H., M.C., K.F.C., J.V.F., A.M.F., E.I., N.N.J., B.L., D.T.M., W. C.M., P.N., S.P.P., W.G.T., S.E.W., S.C.E., and N.S. designed research; J.Z., B.G., P.B., M.D.D., S.C., N.V.M., C.C., M.P., M.A., W.X., W.W.B., S.C.E., and N.S. performed research; J.Z., W.J.C., V.O., G.A.H., M.C., K.F.C., J.V.F., A.M.F., E.I., N.N.J., B.L., D.T.M., W. C.M., P.N., S.P.P., W.G.T., S.E.W., S.C.E., and N.S. contributed new reagents/analytic tools; J.Z., B.G., P.B., M.D.D., R.P.I., E.R.B., D. Myers, S.C., N.V.M., M.P., M.A., W.W.B., W.J.C., V.O., G.A.H., M.C., K.F.C., J.V.F., A.M.F., E.I., N.N.J., B.L., D.T.M., W. C.M., P.N., S.P.P., W.G.T., S.E.W., S.C.E., and N.S. analyzed data; and J.Z., B.G., S.C.E., and N.S. wrote the paper.

Competing interest statement: Cleveland Clinic has applied for patents on HSD3B1. This article is a PNAS Direct Submission. This open access article is distributed under Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND).

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This article contains supporting information online at https://www.pnas.org/lookup/suppl/ doi:10.1073/pnas.1918819117/-/DCSupplemental.

First published January 13, 2020.
levels of adrenal and gonadal androgens in males and females during adolescence are associated with improving asthma during adolescence (12). However, the role of GC-induced androgen suppression in the pathophysiology of severe, GC-resistant human asthma is not established.

The androgen dehydroepiandrosterone (DHEA) and its sulfate (DHEA-S) are secreted from the adrenal reticularis (13, 14), and together are the most abundant steroid in circulation. However, its function is not known. DHEA-S may have an immunomodulatory effect, but clinical results have been inconsistent, possibly because variations in DHEA-S metabolism are generally not taken into account (15). In peripheral tissues, DHEA is metabolized by the enzyme 3β-hydroxysteroid dehydrogenase-1 (3β-HSD1; encoded by HSD3B1) to potent downstream androgens (e.g., testosterone and dihydrotestosterone). A common missense-encoding variant in HSD3B1, rs1047303 (c.1245C > A, p.T367N), regulates biochemical function and clinical phenotypes. The HSD3B1 (1245A) allele encodes for an adrenal restrictive enzyme that limits conversion from DHEA to downstream androgens, whereas the HSD3B1 (1245C) allele encodes for an adrenal permissive enzyme that results in increased metabolic flux from DHEA to more potent androgens (16). Multiple studies from the United States, Japan, and Spain, now further confirmed in a phase 3 clinical trial, clearly show that men with advanced prostate cancer treated with castration who inherit the adrenal permissive 3β-HS1 allele, which confers more rapid conversion of DHEA to potent androgens, have a greater end stage of androgen-driven disease progression (17–22). These studies establish that clear clinical phenotypes are associated with HSD3B1 genotypes that biochemically confer fast and slow metabolic flux to potent androgens. We hypothesized that the restrictive HSD3B1 genotype that enables DHEA-S conversion to potent androgens impairs FEV1PP specifically when GC treatment suppresses adrenal DHEA-S production, limiting substrate availability for 3β-HSD1 and possibly providing a mechanistic explanation for GC-resistant severe asthma in patients with this genotype.

Severe Asthma Research Programs (SARP) are comprehensive networks sponsored by the National Institutes of Health/ National Heart, Lung, and Blood Institute that aimed to characterize and understand the pathobiology of severe asthma. Here, we determined FEV1PP in the SARP III cohort for patients with asthma treated with GC and without (noGC) daily oral GCs. Confirmatory studies were performed in patients with severe asthma from the SARP I&II study.

Methods

Subjects. Individuals included in this analysis comprised a subset of 318 Caucasian (from 488 patients of all races) adults (aged >18 y) with asthma enrolled in the SARP III study for whom genotyping data and pulmonary function testing were available. We only included Caucasian patients in our study because of the numbers of patients available for analysis and the higher adrenal permissive HSD3B1 (1245C) allele frequency of 30% to 40% in Caucasians, as opposed to ~8% in people of African descent.

SARP III, a network of 11 clinical research centers across the United States, recruited 713 participants with asthma between 1 November 2012, and 1 October 2015. Of those 713 initially recruited participants with asthma, 526 were adults (aged >18 y), 60% had severe asthma as defined by the European Respiratory Society/American Thoracic Society criteria (3), and 17% (21% of adult Caucasians) were chronically treated with chronic oral and/or injectable GCs (i.e., systemic). The remaining 40% of patients with asthma did not meet the criteria for severe asthma and were classified as having nonsevere asthma. Among the 89 (17%) adult subjects treated with systemic GCs in SARP III, 55 received daily oral GCs only, 15 received daily oral GCs with additional GC injections, and 19 received intermittent GC injections alone. In the group that received intermittent GC injections alone, the majority (14 participants) received 1 to 3 injections per year. The rest (5 subjects) received more than 6 injections per year. While the suppressive effect of GCs is predictable with chronic daily GC use, intermittent GC injections might have an inconsistent effect on adrenal suppression, and accordingly, participants receiving such therapy were excluded from the analysis. Patients were also excluded if they were pregnant or breast-feeding during the initial characterization period, had a history of premature birth (<35 wk gestation), had a diagnosis of any other chronic pulmonary disorder. All patients were not active smokers (past smokers should not have smoked within a year or exceeded 10 pack-years of tobacco use if >30 y of age, or <5 pack-years if <30 y of age) and were required to have evidence of bronchial hyperresponsiveness (defined as a PC20 methacholine value <16 mg/mL) or reversible airflow obstruction, as evidenced by an increase in FEV1 of 12% or greater after albuterol inhalation, ipratropium bromide inhalation, or both. Spirometry was performed according to the American Thoracic Society/European Respiratory Society guidelines (23, 24). The 2012 Global Lung Initiative standard reference equations were used to predict spirometric reference values (25). DHEA-S levels were analyzed at the University of Virginia Center for Research in Reproduction Lindag Core Laboratory, using the Siemens Immulite 2000 immunoassay system, which has a lower limit of detection for DHEA-S of 15 µg/dL. Information on SARP III network, protocol, and characterization procedures have been published previously (26–28). All participants provided written informed consent. The institutional review board at each center approved the study. The study is listed on ClinicalTrials.gov.

To replicate primary findings, 184 Caucasian participants (from a total of 263 of all races) with severe asthma were selected from the SARP I&II cohorts. Similarly, SARP I&II is a National Institute of Health/National Heart, Lung, and Blood Institute-sponsored multicenter study that recruited patients with asthma between 2001 and 2012 from 9 sites in the United States and 1 in the United Kingdom. However, as compared with SARP III, patients with asthma enrolled in SARP I&II were less likely to have severe asthma (40% vs. 60%) (28–30). In contrast to SARP III, severe asthma was defined in SARP I&II according to the initial ATS workshop definition of severe asthma (31).

Statistical Analysis. Whole-genome sequence of 1,888 patients enrolled in SARP I, II, and III was released by the Trans-Omics for Precision Medicine (TOPMed) program (http://www.nhlbiwgs.org/) in its genotype call sets freeze 6a version. Whole-genome sequence with a read depth of 38X was performed on blood DNA, using Illumina HiSeq X technology. The TOPMed freeze 6a genotype call set includes 107,047 samples and 642M high-quality variants genomewide, >7.6M coding variants, and >350,000 loss-of-function variants (http://top.med.ohio-state.edu). Genotype calling and quality control were performed by the Informatics Research Center, led by the University of Michigan.

Genotypes for variant rs1047303 (position chr1:119514623 [build GRCh38.p12]) in SARP I, II, and III were extracted with PLINK2 (32, 33) (https://www.cog-genomics.org/plink2). HSD3B1 genotypes were directly confirmed in 28 patients, using a method previously validated with 100% match (17).

Student’s t test was used for 2 group comparisons of continuous normally distributed variables. The means of the 3 different genotype groups were compared using the ANOVA test. Pairwise comparisons were performed using the Tukey-Kramer Honest Significant Differences (HSD) test. Otherwise, Wilcoxon’s rank sum test or Kruskal–Wallis one-way ANOVA were used when normality assumptions were not met. Categorical variables were compared using a χ2 test. We assumed an additive model of inheritance, in which each variant allele was an additive effect for the rs1047303 genotype (coded as the number of C alleles). Interaction terms were included between rs1047303 genetic variants and daily oral GC as the dependent variables of the prebronchodilator FEV1PP, (pre-FEV1PP) post-FEV1PP. Models were fit under the assumption of a normal distribution for FEV1PP. All statistical analyses were conducted with R, version 3.5.3 (34) (Project for Statistical Computing, Vienna, Austria). A P value < 0.05 was considered statistically significant because only one position was tested.

Data Availability. TOPMed genomic data and preexisting parent study phenotypic data are made available to the scientific community in study-specific accessions in the database of Genotypes and Phenotypes (https://www.ncbi.nlm.nih.gov/gap/).

Results

Baseline Characteristics. Baseline characteristics of Caucasian SARP III participants with asthma and SARP I&II participants with severe asthma are listed in Table 1. Aside from higher body mass index (BMI) in participants with the CC compared with the AA genotype enrolled in SARP III, patients with the AA, AC, and CC genotypes had similar baseline characteristics. Other than the CC genotype being associated with higher FEV1/FVC ratio compared with AA and AC, all other characteristics were not significantly different in participants with severe asthma enrolled in SARP I, II, or III.
Baseline DHEA-S and FEV_{1PP}. FEV_{1PP} was weakly associated with serum DHEA-S in both men and women. In the SARP III cohort, the $R^2$ (proportion of FEV_{1PP} variability explained by DHEA-S) was 0.04 for all races ($n = 314; P < 0.001$) and 0.04 ($n = 203; P < 0.001$) for Caucasians. Similarly, for severe asthma in SARP I&II, the $R^2$ was 0.13 ($n = 271; P < 0.001$) for all races and 0.20 ($n = 178; P < 0.001$) in Caucasians (Fig. 1A and SI Appendix, Table S1). This association appears to be driven by patients with low DHEA-S (i.e., first quartile; SI Appendix, Fig. S1). Strikingly, no women and very few men with DHEA-S levels over 200 μg/dL in either cohort had a baseline FEV_{1PP} of less than 75% in SARP III.

DHEA-S Suppression Is Associated with Oral GC Use. Daily oral GC therapy was commonly used in SARP III with median dose and duration that did not differ among the 3 HSD3B1 genotypes. The median duration was 12 mo for each of the 3 genotypes ($P = 0.18$ by Kruskal–Wallis test), and the median dose was 10 mg prednisone ($P = 0.74$ by Kruskal–Wallis test). Overall, 22.6% (29% of Caucasians) of adults with severe asthma enrolled in SARP III were treated with daily oral GC therapy (26) (SI Appendix, Table S2). Endogenous circulating cortisol declined significantly ($P < 0.001$) in patients receiving oral GCs, confirming both patient compliance and adrenal suppression (SI Appendix, Fig. S2). As expected, our analysis of DHEA-S from 314 adult participants with asthma enrolled in SARP III showed significantly lower plasma DHEA-S levels in patients treated with daily oral GC therapy, as opposed to those not receiving daily oral GCs for both men and women. Not surprisingly, DHEA-S decline occurs with asthma enrolled in SARP III and SARP I&II ($P < 0.001$) (SI Appendix, Table S3). Circulating DHEA also decreased by about 70% with GC therapy (SI Appendix, Fig. S5).

HSD3B1(1245) Genotype and GC Resistance. To test our hypothesis that the HSD3B1(1245A) adrenal restrictive allele is specifically associated with impaired lung function with GC treatment-mediated adrenal suppression, lung function was compared in 318 GC and noGC Caucasian patients enrolled in SARP III for whom HSD3B1(1245) genotype data were available. Statistical comparisons of GC and noGC FEV_{1PP} by HSD3B1(1245) genotype, before and after bronchodilator (BD), are summarized in Table 1.
The mechanisms by which androgens can benefit asthma have been described in detail in animal models. In particular, androgens directly inhibit airway inflammation (4, 8, 9, 15), airway smooth muscle, and fibroblast proliferation (4–7). Consistent with these data, we have recently shown that DHEA therapy improves lung function in low-DHEA-S women (34). Moreover, our additional analysis shows that all women in our study whose serum DHEA-S increased by more than 300 μg/dl had an increase in FEV1 (n = 8) compared with those who had less of an increase in DHEA-S (P = 0.018 by Fisher’s exact test). Furthermore, the plausibility of DHEA/DHEA-S benefit in asthma is further supported by 3β-HSD1 expression in the human lung (SI Appendix, Fig. S10). A model that summarizes the effect of the association between HSD3B1(1245) genotype, DHEA-S suppression with GC treatment and FEV1PP is shown in Fig. 3.

Discussion

Severe asthma is defined as asthma that remains symptomatic and exacerbation-prone despite controlled high-dose inhaled ICS or systemic steroid treatment in conjunction with a second controller medication (3). Causes underlying severe asthma are heterogeneous (28, 30, 35, 36), and many patients are refractory, even to recently developed biological therapies (36). An aspect of severe asthma that is not commonly considered is that systemic GC therapy increases risk for low circulating levels of androgens, particularly DHEA-S (37).

Our study supports a model in which HSD3B1(1245) genotypes that confer less active conversion from adrenal precursors to potent androgens in peripheral tissues leads to a physiologic state of relative androgen deficiency that occurs specifically with DHEA-S suppression that is a consequence of systemic GC treatment. Strikingly, an HSD3B1(1245) allele-dose dependent effect appears to be clear and occurs for pre-BD-FEV1PP and post-BD-FEV1PP in both SARP III and SARP I&II.

In general, androgens require the AR to mediate much of their physiologic effects. Potent AR stimulation from adrenal DHEA/DHEA-S, which is available in circulation, requires enzymatic conversion by 3β-HSD1 to testosterone and dihydrotestosterone, which occurs in peripheral tissues. Given the variety of tissues in which AR is expressed, the association observed in our study may be attributable to androgen stimulation in several different cell types. Androgens have many effects that could be beneficial for the asthmatic airway. For example, DHEA-S inhibits human airway smooth muscle and fibroblast proliferation and may benefit airway epithelial to mesenchymal transition (4–6). Both DHEA-S

Table 2. Comparison of maximum post-BD-FEV1,% between patients treated with and without daily oral GCs among the HSD3B1(1245) AA, AC, and CC genotypes*  

| HSD3B1(1245) Genotype | SARP III Not on GC | On GC | P value | SARP I & II Not on GC | On GC | P value |
|------------------------|--------------------|------|---------|-----------------------|------|---------|
| AA                     |                    |      |         |                       |      |         |
| n                      | 123                | 87   |         |                       | 45   | 35      |
| Pre-FEV1, % of predicted value | 75.1 ± 18.3 | 54.3 ± 15.3 | <0.001 | 63.4 ± 18.8 | 49.8 ± 17.1 | 0.001 |
| Post-FEV1, % of predicted value | 83.9 ± 18.8 | 64.2 ± 19.6 | <0.001 | 73.5 ± 15.9 | 63.7 ± 18.4 | 0.024 |
| AC                     |                    |      |         |                       |      |         |
| n                      | 107                | 87   |         |                       | 45   | 49      |
| Pre-FEV1, % of predicted value | 75.6 ± 21.6 | 59.4 ± 19.8 | <0.001 | 66.9 ± 22.3 | 54.3 ± 18.3 | 0.008 |
| Post-FEV1, % of predicted value | 83.2 ± 20.5 | 67.7 ± 17.9 | <0.001 | 77.2 ± 20.8 | 67.8 ± 20.3 | 0.07 |
| CC                     |                    |      |         |                       |      |         |
| n                      | 33                 | 8    |         |                       | 15   | 10      |
| Pre-FEV1, % of predicted value | 78.9 ± 16.3 | 73.4 ± 16.6 | 0.41 | 67.7 ± 22.5 | 66.7 ± 26.6 | 0.92 |
| Post-FEV1, % of predicted value | 89.2 ± 17.0 | 83.1 ± 14.4 | 0.32 | 78.2 ± 22.3 | 80.7 ± 16.2 | 0.76 |

*Data are presented as mean ± SD for continuous variables and proportions or percentages for categorical variables.

1P value comparing daily oral GC vs. no GC in each genotype group AA, AC, and CC.
and testosterone promote airway smooth muscle relaxation (7). Testosterone is associated with decreased Th2 and Th1 inflammation in animal models of asthma (8, 9). Epidemiologically, androgens are associated with better lung function in large healthy cohorts (10, 11) and in disease (12). Increasing circulating levels of adrenal and gonadal androgens in males and females during adolescence are believed to be associated with improving asthma during adolescence (12), and gonadal androgens in particular may be associated with gender-based differences in asthma incidence and severity in adulthood (12, 38–40). Notably, steroid metabolites downstream of DHEA-S are generated at the level of the target peripheral tissue and are generally not appreciable in circulation (41, 42). These data suggest that androgen depletion, whether circulating and/or at the tissue level, could contribute to the pathophysiology

![Fig. 2.](image)

**Fig. 2.** The adrenal restrictive HSD3B1(1245A) allele is specifically associated with poor pulmonary function in GC-treated patients with severe asthma. In Caucasian AA genotype patients with asthma enrolled in SARP III, baseline prebronchodilator FEV1/PP (Pre-BD FEV1/PP) (A) and postbronchodilator FEV1/PP (Post-BD FEV1/PP) (B) is lower for those in the GC vs. no GC treatment groups. In contrast, for the CC genotype, there is no difference between GC and no GC treatment groups. Lower Pre-BD FEV1/PP (C) and Post-BD FEV1/PP (D) for AA genotype patients receiving GC also occurs in Caucasian patients with severe asthma enrolled in SARP I&II. Error bars indicate SEs.

![Fig. 3.](image)

**Fig. 3.** A model that explains physiologic effects of HSD3B1 inheritance on FEV1/PP in patients with severe asthma. GC treatment suppresses adrenal DHEA, which may become a limiting substrate for 3β-HSD1, depending on HSD3B1 genotype. Adrenal permissive and adrenal restrictive alleles enable and limit metabolic flux through 3β-HSD1 and recovery of airflow.
of severe asthma and resistance to oral GC therapy. Our data confirm that low DHEA-S levels in asthma are associated with low lung function. However, we do not know whether this is a causal relationship, whether low androgen levels are simply a marker associated with patients with low lung function on more GCs, or both. Recently we published a pilot study that suggests DHEA supplementation in women with low DHEA-S improves FEV1 (34).

In recent studies of prostate cancer, a recent study by Lanctot et al. demonstrated that the adrenal restrictive HSD3B1(1245A) allele limits conversion of DHEA to more potent androgens, whereas the adrenal permissive HSD3B1(1245C) allele increases tissue production of potent androgens (16, 17). The SARP studies provided an opportunity to study androgen metabolism effects in severe asthma. We therefore hypothesized that the restrictive allele, which impedes conversion from adrenal DHEA-S to testosterone and dihydrotestosterone, would be associated with lower lung function in severe asthma when systemic GCs are used and suppress substrate availability for 3p-HSD1. Data were analyzed in the SARP III cohort, enriched for severe asthma, and then validated in the patients with severe asthma in the SARP I&II cohort and are strikingly consistent with our hypothesis across both cohorts.

Limitations of this study include that it is restricted to Caucasian patients with asthma and the sample size, particularly for patients with severe asthma, is small. For example, only 1 African American participant with CC genotype was enrolled in SARP III, and 2 others in SARP I&II. The lower prevalence of the adrenal permissive C allele in African Americans could contribute to higher severity and risk from asthma, as well as GC resistance, in this patient population. Similarly, none of the 27 adult Hispanic participants with asthma enrolled in SARP III, and only 1 of the 34 adult Hispanics enrolled in SARP I&II, respectively, carried the CC genotype. Of note, the adrenal permissive allele frequency (i.e., the C allele) was 36% and 37% in adult Caucasian participants with asthma enrolled in SARP III and severe asthma enrolled SARP I&II. Those frequencies match the frequency reported for Caucasians in the 1000 Genomes Project and suggest the absence of any deviation from Hardy–Weinberg Equilibrium.

In conclusion, we have identified a genetic determinant associated with GC resistance and low lung function in asthma. Evidence that the adrenal restrictive HSD3B1(1245A) allele, which confers a lower level of prostate cancer adrenal androgen dependence by preventing local conversion of DHEA to more potent androgens, adversely affects lung function in GC-dependent severe asthma suggests that androgens have a central role in the pathophysiology of human severe asthma and response to systemic GC treatment. Our data provide genetic evidence that complements epidemiological data regarding a benefit of androgens on lung function. Our model is further supported by our data showing a positive relationship between circulating adrenal DHEA-S levels and lung function. Indeed, androgens cause airway smooth muscle relaxation and prevent both remodeling and inflammation in animal models of asthma. GC use suppresses endogenous androgen production, preventing the beneficial effects of androgens in human asthma. These data suggest the possibility that HSD3B1 genotype is predictive of which patients might benefit from systemic GC therapy alone and, for those who are resistant, who would benefit from androgen replacement in severe asthma.

ACKNOWLEDGMENTS. We thank the patients and their families for their participation in the Severe Asthma Research Program; the site-based study coordinators and regulatory personnel for enrolling participants, the Data Safety and Monitoring Board for program oversight, and National Institute of Health/National Heart, Lung, and Blood Institute project officers and staff who administered the protocol. Supported by a grant from the National Heart, Lung, and Blood Institute Severe Asthma Research Program (U10 HL109250, P01 HL128192, and P01 HL108171 to Rainbow Babies and Children’s Hospital Virginia-Cleveland Consortium, and R01 HL69170, U10 HL109250, K08 HL133381, P01 HL103453, P01 HL81064, R01CA172382, R01CA190289, R01 CA236780, and a grant from the Prostate Cancer Foundation to the Cleveland Clinic). We also thank the Harrington Discovery Institute of University Hospitals, Cleveland, for grant support.
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