data support the hypothesis that the relationship between improved asthma control scores among beclomethasone-treated children with the *CYP3A5*1D/*1D* genotype is ultimately due to the inactivation of CYP3A5 caused by the *CYP3A5*3* SNP.

The physiological basis for the association between improved asthma control with beclomethasone and *CYP3A5*3* is not completely understood. This inactivating SNP abolishes CYP3A5 activity both in the lung and in the liver.[8] Reduced pulmonary and hepatic enzyme activity is likely to prolong the presence of active beclomethasone within the airway, thereby increasing the duration of its anti-inflammatory effects. In addition, further investigation is warranted to determine whether diminished CYP3A5 activity may be associated with higher systemic concentrations of beclomethasone, which has the potential to increase the risk of adverse effects, including suppression of the hypothalamic-pituitary-adrenal axis.

Interpretation of our findings should be considered in light of several limitations. First, the precision of our effect estimates is limited by our sample size (n = 64). Second, it was not possible to directly measure CYP3A5 expression or tissue-specific activity; however, these studies are ongoing. Last, we did not obtain pulmonary function tests because standard spirometry measurements require a degree of patient cooperation that is difficult to achieve in the youngest of children.

The clinical relevance of this observed association requires further mechanistic explanation and additional study with larger sample sizes. Nevertheless, these data support an association between improved asthma control with inhaled beclomethasone and the loss of function *CYP3A5*3* allele and are consistent with our earlier work in which asthma control was found to be improved among children treated with fluticasone who had a genotype consistent with reduced CYP3A4 activity.[4] When genetic testing is clinically available, these findings may be useful in selecting an appropriate therapeutic agent for patients who do not achieve optimal control with their currently prescribed inhaled glucocorticoid.

We thank Bradley W. Thomas and Amber Bagherian for their technical support.

*Chris Stockmann, MSc*[a,b]
*Christopher A. Reilly, PhD*[b]
*Bernhard Fassl, MD*[a]
*Roger Gaedigk, PhD*[c]
*Flory Nkoy, MD, MPH, MS*[a]
*Bryan Stone, MD*[a]
*Jessica K. Roberts, PhD*[a]
*Derek A. Uchida, MD*[a]
*J. Steven Leeder, PharmD, PhD*[c]
*Catherine M. T. Sherwin, PhD*[a]
*Michael G. Spigarelli, MD, PhD*[a]
*Garold S. Yost, PhD*[b]†
*Robert M. Ward, MD*[a,b]

From [a]the Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, Utah; [b]the Department of Pharmacology/Toxicology, University of Utah College of Pharmacy, Salt Lake City, Utah; and [c]the Division of Clinical Pharmacology, Toxicology and Therapeutic Innovation, Children's Mercy Hospital, Kansas City, Mo. E-mail: Robert.Ward@hsc.utah.edu.

†Deceased.

This project was supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (grant no. R01 HD060559). C.S. is supported by the American Foundation for Pharmaceutical Education's Clinical Pharmaceutical Sciences Fellowship. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Eunice Kennedy Shriver National Institute of Child Health and Human Development or the National Institutes of Health. Additional support was provided by the Primary Children's Medical Center Research Foundation and the Center for Clinical and Translational Sciences (grant no. CTSA 5UL1RR025764-02).

Disclosure of potential conflict of interest: C. Stockmann has received research support from the National Institutes of Health-Eunice Kennedy Shriver National Institute of Child Health and Human Development and the American Foundation for Pharmaceutical Education. F. Nkoy has received research support from the National Institutes of Health-Eunice Kennedy Shriver National Institute of Child Health and Human Development and the Patient-Centered Outcomes Research Institute (51002874) and has a pending patent application for electronic Asthma Tracker. C. A. Reilly, B. Fassl, R. Gaedigk, B. Stone, J. K. Roberts, D. A. Uchida, J. S. Leeder, C. M. T. Sherwin, M. G. Spigarelli, and R. M. Ward have received research support from the National Institutes of Health-Eunice Kennedy Shriver National Institute of Child Health and Human Development.

## REFERENCES

1. 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.
2. Szefler SJ, Phillips BR, Martinez FD, Chinchilli VM, Lemanske RF, Strunk RC, et al. Characterization of within-subject responses to fluticasone and montelukast in childhood asthma. J Allergy Clin Immunol 2005;115:233-42.
3. Drazen JM. A step toward personalized asthma treatment. N Engl J Med 2011;365:1245-6.
4. Stockmann C, Fassl B, Gaedigk R, Nkoy F, Uchida DA, Monson S, et al. Fluticasone propionate pharmacogenetics: CYP3A4*22 polymorphism and pediatric asthma control. J Pediatr 2013;162:1222-7, 1227.e1-2.
5. Wang D, Guo Y, Wrighton SA, Cooke GE, Sadee W. Intronic polymorphism in CYP3A4 affects hepatic expression and response to statin drugs. Pharmacogenomics J 2011;11:274-86.
6. Roberts JK, Moore CD, Ward RM, Yost GS, Reilly CA. Metabolism of beclomethasone dipropionate by cytochrome P450 3A enzymes. J Pharmacol Exp Ther 2013;345:308-16.
7. National Asthma Education and Prevention Program. 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.
8. 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. Nat Genet 2001;27:383-91.
9. 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 2006;36:1191-200.
10. Hedrick PW. Gametic disequilibrium measures: proceed with caution. Genetics 1987;117:331-41.

Available online March 29, 2015.
http://dx.doi.org/10.1016/j.jaci.2015.02.009

## Whole-exome sequencing of Ethiopian patients with ichthyosis vulgaris and atopic dermatitis

*To the Editor:*

Ichthyosis vulgaris (IV) is a common skin disease characterized by palmar hyperlinearity, excess scaling, and keratosis pilaris. The underlying genetic cause of IV is haploinsufficiency of the filaggrin gene *(FLG)* because of loss-of-function mutations,[1] leading to early termination of profilaggrin and loss of filaggrin expression. Atopic dermatitis (AD) is a common complex inflammatory skin disorder with a high prevalence (20%) among children of Western countries.[2] In Ethiopia, contrasting frequencies of AD have been reported, ranging from 1.2% to 19%.[2] AD and IV show phenotypically overlapping features. The most significant genetic susceptibility factor to AD in European populations is truncating loss-of-function mutations of *FLG*, although with a reduced penetrance compared with IV.[1] Even though few studies have been performed in African

**TABLE I.** Variants identified in EDC locus genes

| Chromosome | Position (hg19) | Gene | Nucleotide and amino acid change | Type of variation | MAF in 1000G (African) | MAF in ExAC (African) | dbSNP no. |
|---|---|---|---|---|---|---|---|
| 1 | 152085074 | *TCHH* | NM_007113:exon3:c.619G>T:p.E207X | Nonsense | — | — | — |
| 1 | 152059278 | *TCHHL1* | NM_001008536:exon3:c.880C>T:p.Q294X | Nonsense | 0.0204 | 0.0160 | rs61749316 |
| 1 | 152323132 | *FLG2* | NM_001014342:exon3:c.7130C>A:p.S2377X | Nonsense | — | — | rs12568784 |
| 1 | 152383382 | *CRNN* | NM_016190:exon3:c.G176A:p.R59H | Missense | 0.0106 | 0.0056 | rs145120458 |
| 1 | 152191822 | *HRNR* | NM_001009931:exon3:c.A2283C:p.Q761H | Missense | — | — | — |
| 1 | 152191419 | *HRNR* | NM_001009931:exon3:c.C2686T:p.R896C | Missense | — | — | — |
| 1 | 152190027 | *HRNR* | NM_001009931:exon3:c.C4078T:p.P1360S | Missense | — | — | — |
| 1 | 152192605 | *HRNR* | NM_001009931:exon3:c.G1500C:p.Q500H | Missense | — | — | — |
| 1 | 152192541 | *HRNR* | NM_001009931:exon3:c.T1564A:p.S522T | Missense | — | — | — |

A complete list of genes is given in Table E1.

*CRNN*, Cornulin; *dbSNP*, Single Nucleotide Polymorphism database; *ExAC*, Exome Aggregation Consortium; *FLG2*, filaggrin family 2; *1000G*, 1000 Genomes Project; *HRNR*, hornerin; *TCHH*, trichohyalin; *TCHHL1*, trichohyalin-like 1.

**TABLE II.** Variants identified in non-EDC locus genes

| Chromosome | Position (hg19) | Gene | Nucleotide and amino acid change | Type of variation | MAF in 1000G (African) | MAF in ExAC (African) | dbSNP no. |
|---|---|---|---|---|---|---|---|
| 20 | 3654244 | *ADAM33* | NM_001282447:exon10:c.G967T:p.E323X | Nonsense | — | — | — |
| 18 | 28971048 | *DSG4* | NM_001134453:exon7:c.G692A:p.S231N | Missense | — | — | — |
| 18 | 28968349 | *DSG4* | NM_001134453:exon4:c.C236T:p.S79L | Missense | 0.0061 | 0.0046 | rs36040686 |
| 18 | 28979427 | *DSG4* | NM_001134453:exon9:c.G1198A:p.G400R | Missense | 0.0061 | 0.0045 | rs35378785 |
| 17 | 74004853 | *EVPL* | NM_001988:exon22:c.C4433T:p.T1478M | Missense | — | — | — |
| 17 | 74003539 | *EVPL* | NM_001988:exon22:c.C5747T:p.S1916L | Missense | — | — | — |
| 6 | 158613020 | *GTF2H5* | NM_207118:exon3:c.T47G:p.M16R | Missense | — | — | — |
| 17 | 5462548 | *NLRP1* | NM_001033053:exon4:c.T1468G:p.F490V | Missense | — | — | — |
| 5 | 147449998 | *SPINK5* | NM_001127698:exon3:c.C194T:p.T65M | Missense | — | — | — |
| 5 | 147469114 | *SPINK5* | NM_001127698:exon7:c.G532A:p.E178K | Missense | — | 0.0001 | — |
| 5 | 147480955 | *SPINK5* | NM_001127698:exon14:c.A1258G:p.K420E | Missense | 0.1989 | 0.2433 | rs2303067 |
| 5 | 147481363 | *SPINK5* | NM_001127698:exon15:c.G1322A:p.R441H | Missense | 0.0219 | 0.0243 | rs34393923 |
| 5 | 147496004 | *SPINK5* | NM_001127698:exon22:c.2087_2089del: p.696_697del | Nonframeshift deletion | 0.0219 | 0.0238 | rs111662216 |

A complete list of genes is given in Table E2.

*ADAM33*, ADAM metallopeptidase domain 33; *dbSNP*, Single Nucleotide Polymorphism Database; *DSG4*, desmoglein 4; *EVPL*, envoplakin; *ExAC*, Exome Aggregation Consortium; *1000G*, 1000 Genomes Project; *GTF2H5*, general transcription factor IIH, polypeptide 5; *NLRP1*, NLR family, pyrin domain containing 1; *SPINK5*, serine peptidase inhibitor, Kazal type 5.

populations, we and others reported either absence or very low frequency of *FLG* mutations, suggesting another cause for IV/AD.[2-4] In contrast, a recent publication by Polcari et al[3] reported *FLG* mutations in 22.2% of African Americans with IV/AD, but here the possibility of genetic admixture must be taken into account.

Given previous findings, in this study we set out to identify other underlying genetic factors in this population.[2] Assuming that IV is a monogenic disease with an effect on AD susceptibility, we selected 22 Ethiopian patients (18 unrelated subjects and 2 parent-child pairs) manifesting both IV and AD for whole-exome sequencing (WES). The diagnosis was based on clinical findings done by the same dermatologist (K.D.B.), and the UK Working Party's diagnostic criteria were applied for AD. All patients were recruited in Gondar, Ethiopia. Their median age was 9 years (range, 1-31 years), 50% were female, and 73% had severe AD (SCORAD score >40). All patients had keratosis pilaris, palmar hyperlinearity, and xerosis and had negative results for *FLG* mutations, as reported previously.[2] A larger IV/AD cohort (n = 155) and matched control subjects (n = 192) from the same region were included to evaluate the frequencies of 7 single nucleotide variants (SNVs) identified in patients undergoing WES.

WES was performed at SciLifeLab (Stockholm, Sweden). Libraries were prepared with the SureSelect Human All Exon V4 or V5 kit (Agilent Technologies, Santa Clara, Calif) and sequenced on the HiSeq 2000 (Illumina, San Diego, Calif) to a minimum sequence depth of 87×. The data were analyzed with in-house pipelines. A minor allele frequency (MAF) of 5% for African populations was applied to the 1000 Genomes Project (1000Goct2014) and the Exome Aggregation Consortium (ExAC 0.2) data set to exclude common variants. Less stringent filtering parameters were additionally applied to identify relatively common nonsense variants. On average, each patient showed 1364 (1234-1486) nonsynonymous exonic variants. Seven SNVs were assayed by using Custom TaqMan SNP Genotyping Assays (Life Technologies, Grand Island, NY) with a QuantStudio 7 FLEX instrument (Life Technologies). Significance was evaluated by using the Fisher exact test ($P \leq .05$ was considered significant).

In accordance with our previous Sanger sequencing data, we did not identify any loss-of-function mutations in *FLG*. The coverage of the coding part was almost complete, with a read depth of between 61× and 296×.

We were particularly interested in genes clustered on chromosome band 1q21 (epidermal differentiation complex [EDC])