Patients with Mutations in Gsα Have Reduced Activation of a Downstream Target in Epithelial Tissues due to Haploinsufficiency

Stephanie C. Hsu, Joshua D. Groman, Christian A. Merlo, Kathleen Naughton, Pamela L. Zeitlin, Emily L. Germain-Lee, Michael P. Boyle, and Garry R. Cutting

Departments of Pediatric Endocrinology (S.C.H., E.L.G.-L.), Pulmonary and Critical Care Medicine (C.A.M., M.P.B.), and Pediatric Pulmonology (P.L.Z.) and Institute of Genetic Medicine (J.D.G., K.N., G.R.C.), Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Context: Patients with Albright hereditary osteodystrophy (AHO) have defects in stimulatory G protein signaling due to loss of function mutations in GNAS. The mechanism by which these mutations lead to the AHO phenotype has been difficult to establish due to the inaccessibility of the affected tissues.

Objective: The objective of the study was to gain insight into the downstream consequences of abnormal stimulatory G protein signaling in human epithelial tissues.

Patients and Design: We assessed transcription of GNAS and Gsα-stimulated activation of the cystic fibrosis transmembrane conductance regulator (CFTR) in AHO patients, compared with normal controls and patients with cystic fibrosis.

Main Outcome Measures: Relative expression of Gsα transcripts from each parental GNAS allele and cAMP measurements from nasal epithelial cells were compared among normal controls and AHO patients. In vivo measurements of CFTR function, pulmonary function, and pancreatic function were assessed in AHO patients.

Results: GNAS was expressed equally from each allele in normals and two of five AHO patients. cAMP generation was significantly reduced in nasal respiratory epithelial cells from AHO patients, compared with normal controls (0.4 vs. 0.6, P = 0.0008). Activation of CFTR in vivo in nasal (P = 0.0065) and sweat gland epithelia (P = 0.01) of AHO patients was significantly reduced from normal. In three patients, the reduction in activity was comparable with patients with cystic fibrosis due to mutations in CFTR. Yet no AHO patients had pulmonary or pancreatic disease consistent with cystic fibrosis.

Conclusions: In humans, haploinsufficiency of GNAS causes a significant reduction in the activation of the downstream target, CFTR, in vivo. (J Clin Endocrinol Metab 92: 3941-3948, 2007)

SIGNALING VIA THE stimulatory G protein, Gsα, is critical for normal growth and development (reviewed in Ref. 1). Loss of Gsα function results in early embryonic lethality in mice, and no humans have been identified with deleterious mutations in both copies of GNAS, the gene that encodes exons 1-13 of the α-subunit of Gs (2-4). Gsα is involved in coupling transmembrane receptors to adenyl cyclase to initiate cAMP-dependent downstream effects in a variety of tissues (5). The importance of Gsα-mediated signal transduction is illustrated by patients with Albright hereditary osteodystrophy (AHO). Most AHO patients have decreased signaling via Gsα due to heterozygous loss of function mutations in GNAS. Patients with AHO manifest abnormalities in the bone (short stature, brachydactyly, and sc ossifications) and central nervous system (obesity and mild cognitive deficits) (6). A subset of AHO patients also have endocrine features [resistance to PTH, thyroid stimulating hormone, GH, and occasionally the gonadotropins and glucagon] (7-9). The clinical features of AHO are thought to be the consequence of reduced Gsα signaling that leads to insufficient cAMP production to achieve appropriate activation of downstream effectors (10, 11). Fibroblasts and hematopoietic cells from AHO patients have lower levels of cAMP generation than normal controls, consistent with a defect in the G protein signaling pathway (10, 12-14). However, the in vivo consequences of altered Gsα signaling upon downstream targets have not been assessed in any human subjects.

The mechanism by which defects in Gsα signaling lead to hormone resistance in hormone-responsive tissues is suggested by the observation that AHO patients with hormone resistance have invariably inherited a GNAS mutation from their mother (15-20). Preferential expression of maternal transcripts of Gsα has been shown in thyroid and a few ovarian and pituitary samples from healthy individuals (21-24). These observations led to the hypothesis that hormone resistance in AHO patients is due to absence of Gsα signaling in endocrine tissues (1, 25). Studies of mice with genetic lesions in Gsα have been consistent with this concept (2-4). However, Gsα expression is biallelic in human bone, adipose, adrenal, lung, kidney, liver, skin, muscle, pancreas, thymus, and central nervous system tissue (24, 26, 27), suggesting that the bone and metabolism defects in AHO may be caused by a different mechanism. Chondrocytes from a murine model of AHO display premature hypertrophy in vivo, supporting
a haploinsufficiency mechanism of action in endocrine tissues (28).

Studies of the downstream effects of GNAs mutations in humans are limited due to the relative inaccessibility of endocrine, bone, and central nervous system tissues. In secretory epithelial tissues, Gsα signaling regulates activity of the cystic fibrosis transmembrane conductance regulator (CFTR) (29, 30). Several assays of CFTR function in vitro have been developed as diagnostic tools for cystic fibrosis (CF), an autosomal recessive life-limiting disease due to loss of CFTR function. We assessed in vivo CFTR function in AHO patients to determine whether loss of function of one GNAs gene caused measurable alterations in a downstream target.

Subjects and Methods

Study participants

Fourteen patients (or legal guardians) with GNAs mutations, 20 healthy controls, and eight CF patients gave informed consent. Sixteen of 30 normals and five of 14 AHO patients were heterozygous at rs7121 and included in GNAs expression studies. Demographic details of patient groups compared in this study are summarized in Table 1. AHO patients with hormone resistance were in metabolic control (except for patient 297, who has a history of noncompliance) before participation in this study. AHO patient 213 carried a single CFTR mutation and was excluded from functional CFTR studies. All studies were approved by the Joint Committee on Clinical Investigation of the Johns Hopkins University School of Medicine and conducted between 2001 and 2006.

Nasal epithelial cells

Nasal respiratory epithelial cells (NRECs) were collected after local anesthesia (topical 2% Tetracaine) by gentle scraping of the inferior and lateral surfaces of the inferior turbinate using a sterile curette (Rhinoprobe; Arlington Scientific, Inc., Springville, UT). RNA was extracted using RNA-Beet (Tel-Test, Inc., Friendswood, TX). Cells were cultured in bronchial epithelial growth medium (Lonza Walkersville, Inc., Walkersville, MD) on collagen-coated plastic tissue-culture plates at 37 C, 5% CO2. At confluence, cells were trypsinized and transferred to a collagen-coated 96-well plate at a density of 25,000 cells/well and grown for 4 additional days. Cultured cells were verified as respiratory epithelium using the following criteria: 1) formed tight junctions as demonstrated by immunofluorescent staining for the zona occludens protein-1, 2) expressed human cytokeratin-15 and CFTR RNA transcripts by RT-PCR, and 3) produced mucus when grown on permeable supports with an air-liquid interface.

| TABLE 1. Characteristics of normal, CF, and AHO patient groups* |
|--------------------------|-----------------|-----------------|
|                         | n   | Mean age | M:F              |
|                         |     | (range)  |                 |
| GNAS                    |     |          |                 |
| Normals                 | 16  | 34.7 (23–59) | 9:7              |
| AHO                     | 5   | 32 (20–53) | 1:4              |
| cAMP                    |     |          |                 |
| Normals                 | 17  | 34.2 (23–60) | 10:6             |
| AHO                     | 10  | 20.5 (10–53) | 4:6              |
| CF                      | 2   | 23.5 (22–37) | 2:0              |
| NPD                     |     |          |                 |
| Normals                 | 32  | 30.0 (18–50) | 3.5b             |
| AHO                     | 11  | 26.3 (10–53) | 5:6              |
| CF                      | 16  | 30.4 (19–61) | 10:6             |
| Sweat                   |     |          |                 |
| Normals                 | 30  | 35.6 (17–51) | 15:15            |
| AHO                     | 13  | 25.8 (10–53) | 5:8              |
| CF                      | 29  | 27.7 (11–61) | 14:15            |

a NPD data for normals and sweat rate data for normals and CF were published in previous studies (37, 38).
b Gender data available for 8 of 32 normal NPDs.

GNAS transcript analysis

First-strand cDNA was synthesized from 0.5 μg total RNA from NRECs using Stratascript RT (Stratagene, La Jolla, CA) and olig(dT)18 primers according to the manufacturer’s recommendations. GNAs transcripts were amplified using a faint-labeled sense primer from exon 1 (5'-GAAGGGAACACGGCTCTAC-3') and an antisense primer from exon 3 (5'-GCCTTGCACTGCTATAGA-3') under the following PCR conditions: 95 °C for 6 min, 30 cycles of 95 °C for 30 sec, 57 °C for 30 sec, 72 °C for 1 min, and then 72 °C for 10 min. Alleles were distinguished by digestion with FokI 1 DNA endonuclease for 2 h at 37 °C, separation by capillary electrophoresis, and gene scan analysis (ABI Prism 310 genetic analyzer; Applied Biosystems, Inc., Foster City, CA). Alleles harboring a cystine at nt393 were 76 bp shorter than those with a thymidine due to cleavage by FokI. Plasmids were constructed by blunt-ended ligation of the PCR product from the above reaction into pCR2.1 (Invitrogen, Carlsbad, CA). Plasmids representing four endogenous transcripts of GNAs [containing or missing exon 3 and harboring a C or T at nt393 (rs7121 dbSNP)] were created. To determine whether the C or T allele was in cis with known GNAs mutation, cDNA transcripts were ligated into plasmids and used to transform bacteria. Individual clones were sequenced across exons 2, 11, and 12 and the mutation site to determine phase. A minimum of 20 clones was sequenced from each patient sample.

cAMP generation assay

NRECs in 96-well collagen-coated plates were washed twice in PBS without calcium or magnesium and incubated for 1–2 h in HAM F-12 medium at 37 C, 5% CO2. Cells were treated with isoproterenol or forskolin at 1–500 μM for 15 min at room temperature. Cell lysis and cAMP quantification were performed using the cAMP Bioluminescence immunoassay system (GE Healthcare, Bio-Sciences Corp., Piscataway, NJ). All measurements were performed in quadruplicate. cAMP generation in response to 100 μM isoproterenol or 200 μM forskolin was in the plateau phase of the dose-response curve. Maximal cAMP responses were normalized as the ratio of cAMP generation in response to 100 μM isoproterenol divided by cAMP generation in response to 200 μM forskolin. cAMP was not measured in samples that did not expand in culture or exhibited bacterial or fungal contamination.

Typing CFTR alleles

CFTR mutations were identified using the reverse allele-specific oligonucleotide hybridization technique with reagents (CF Gold 1.0; Roche, Indianapolis, IN).

In vivo assays of CFTR

Nasal potential differences (NPDs) were measured following the published protocols (31, 32). All solutions were warmed to 37 C. Measurements of NPD response to 0.1 mM amiloride hydrochloride in Ringler’s solution of NPD response by measurements in response to amiloride in a low chloride solution, 0.125 mM isoproterenol in amiloride/low chloride, and 0.1 mM ATP in amiloride/low chloride. Baseline potential difference (range -10 to -25 mV) and response to adenine (7 mV) confirming proper positioning of the intact respiratory epithelium throughout the test. Patients with upper respiratory infections or inflamed turbinates were not tested. Isoproterenol responses for normal controls and CF patients were obtained from a multicenter NPD study conducted in 1999–2001 (33) and 16 consecutive CF patients who underwent diagnostic NPD testing at Johns Hopkins in 2003 and whose data were deidentified before inclusion. All methods and reagents were identical between the control groups and AHO patients.

β-Adrenergic stimulated sweat rates were obtained following the protocol of Callen et al. (34). Sweat rates were obtained on the same day as NPD measurements and are reported as the average of the results from each arm. Sweat rates were compared with 30 normal controls and 29 CF patients who were previously tested (34). Sweat chloride measurements were obtained by pilocarpine iontophoresis (35).
Clinical signs of CF disease

Pulmonary function testing (PFT) included spirometry before and after bronchodilator administration, single-breath CO diffusing capacity, and helium lung volume measurement. All PFTs were interpreted by two pulmonologists. Serum amylase and trypsin measurements were performed by the Johns Hopkins Clinical Laboratory, whereas stool samples were sent to Genova Diagnostics (Asheville, NC) for pancreatic elastase 1 measurement (ELISA). Sputum collection was attempted for each AHO patient, with bacterial cultures submitted on all samples.

Statistical analysis

Student’s t test was used to compare patient groups when results followed a normal distribution (total GNAS transcript levels, cAMP measurements). Repeated measurements of cAMP generation was assessed for variability by one-way ANOVA. Mann-Whitney rank sum tests were used to compare NPT and sweat rate results among AHO patients, normal controls, and cystic fibrosis patients. All analyses were performed using the Stata 8 statistical package (StataCorp, College Station, TX) and P < 0.05 was deemed significant.

Results

Expression of Gsa is biallelic in nasal respiratory epithelium

We developed a PCR assay that used a single nucleotide polymorphism in exon 5 of GNAS to quantitate Gsa transcripts from each GNAS gene. To ensure that our assay accounted for the alternative Gsa RNA transcripts, four plasmids containing exons 1–6 of GNAS were constructed (with and without exon 3 and with C or T at nt393). PCR conditions were optimized to yield amplification in the linear range (data not shown). The ratio of plasmids containing C vs. T was varied while keeping the total amount constant. The assay accurately reflected the relative proportions of C- vs. T-bearing plasmids in each mixture (Fig. 1A). Expression of Gsa transcripts in nasal epithelium was assessed using samples from healthy normal subjects who were heterozygous at rs7121 (Fig. 1B). Gsa transcripts in normal controls were set at 1.0. The Fig. 1B shows the results of the assay for 10 normal controls and 10 AHO patients.
were present from each GNAS gene in nearly equal amounts (mean 49.4 ± 4.9%).

Because the expression of Gsa may be different in patients who harbor mutations in the gene, allelic expression was analyzed in nasal respiratory epithelium from AHO patients. Five AHO patients were heterozygous at rs7121. Three of these patients had marked reduction in transcripts from the mutation-bearing allele (patients 205, 206, and 207), likely due to nonsense-mediated decay (Fig. 1C). The other two patients had biallelic expression of Gsa despite their frameshift (patient 200) and missense (patient 213) mutations. Both patients were hormone resistant and demonstrated a severe AHO phenotype including short stature, obesity, and cognitive deficits. Although we did not directly quantify absolute levels of GNAS transcription, no compensatory increase in transcription from the normal GNAS allele was seen in either of these two patients. Therefore, we did not find any evidence of preferential expression of Gsa in nasal respiratory epithelium from normals or AHO patients.

cAMP generation is reduced in nasal respiratory epithelial cells from AHO patients

To examine cAMP generation in response to Gsa signaling, NRECs harvested from study participants were exposed to 100 μM isoproterenol, a β-agonist, or 200 μM forskolin, a direct adenylyl cyclase activator, (Fig. 2A) to produce maximal flux through the Gsa signaling pathway (23- to 183-fold increase in cAMP levels over baseline). To facilitate comparisons, β-agonist stimulated cAMP levels were normalized by forskolin response and reported as maximum cAMP response. NRECs from healthy controls (n = 17) had a mean response of 0.62 ± 0.18 and a range of 0.33–0.97 (Fig. 2B). AHO patients (n = 10) had a significantly lower mean response of 0.38 ± 0.12 and a range of 0.25–0.59 (P = 0.0008; Fig. 2, B and C). NRECs from two patients with CF had responses similar to normal controls. Five controls (129, 113, 128, 119, and 100) and four AHO patients (205, 208, 201, and 210) had repeated nasal epithelial sampling on different days. Repeated measurements were not significantly differ-

**Fig. 2.** cAMP generation is significantly lower in AHO patients, compared with normal controls. Maximum cAMP response was determined by the isoproterenol-stimulated cAMP generation normalized to the forskolin response for each sample. A, Dose response of primary nasal epithelial cells to isoproterenol and forskolin. Results shown are from separate patient samples and are representative of results from at least three patients. B, cAMP response in study participants. Multiple bars for the same individual represent samples harvested on different days. Raw isoproterenol response 2,399–22,373 fM/well. Raw forskolin response 4,707–22,404 fM/well. C, Comparison of cAMP generation between normals and AHO patients. Mean values for each participant are represented by a diamond. Black bars, Mean for each group.
ent by one-way ANOVA with a mean difference of 0.12 between the first and second measurement (p = 0.097). Thus, Gs-stimulated CAMP generation in nasal epithelial cells differs in AHO patients and controls.

In vivo measures of Gsa-stimulated CFTR activation are reduced in AHO patients

NPD testing quantitatively measures β-adrenergic Gsa-mediated CFTR-dependent chloride secretion. NPDs were attempted in 14 AHO patients and acceptable recordings were obtained for 11 patients. Because local environmental conditions may differ between nares, each nare was analyzed separately. Repeated measurements were obtained from five of 11 patients (200, 201, 206, 208, and 214), and 29–78% variability (2–11 mV) was noted between nares in the same individual and 0–79% variability (0–17 mV) in the same individual tested on separate days (Fig. 3A). Despite these individual variations, eight of 13 AHO patients had substantial reductions in β-adrenergic/Gsa responses in at least one nare, similar to that observed in CF patients (Fig. 3A). Nasal isoproterenol responses in AHO patients (21 nares) vs. normal controls (64 nares) were lower as a group (Mann-Whitney rank sum analysis, p = 0.0065; Fig. 3B). CF patients (n = 16; 31 nares) with severe loss-of-function mutations in each CFTR gene had lower responses than AHO patients (p < 0.001).

β-Adrenergic-stimulated sweat rates were obtained in AHO patients as a second in vivo measure of Gsa-mediated CFTR activation. Two of nine patients (patients 210 and 206) who were tested on multiple occasions had sweat rates that varied by more than 50% (Fig. 4A). Multiple sweat rates for the remaining seven patients were not significantly different from each other (mean difference = 0.05 mg per 20 ± 0.71 min). As a group, AHO patients had lower sweat rates than normal controls (Mann-Whitney rank sum test, p = 0.01) and were indistinguishable from CF patients (Fig. 4B). Therefore, AHO patients have reduced in vivo measures of Gsa-mediated CFTR activation, compared with normal subjects, and a subset of AHO patients manifests functional deficits comparable with patients with CF.

AHO patients do not manifest clinical signs of CF disease

Nine AHO patients had at least one in vivo measure of β-adrenergic-stimulated CFTR activation in the same range as patients with CF (Table 2). These nine AHO patients were examined for signs of pulmonary or pancreatic exocrine dysfunction typical of patients with CF. None of the patients had chronic cough, daily sputum production, or pathogenic bacteria in sputum cultures. PFTs revealed that four patients had mild restrictive lung disease, two had obstructive lung disease, and three had normal PFTs. Two AHO patients (200 and 212) reported a history of asthma requiring daily medication including a long-acting β-agonist. Both had PFTs consistent with mild restrictive lung disease and did not respond to bronchodilators. The two patients with obstructive findings on PFTs had no history of asthma. One patient (201) had a low forced expiratory volume expired in 1 sec (FEV1) and forced expiratory volume expired in 1 sec to forced vital capacity ratio. This patient has a history of recurrent pancreatitis as well as Arnold-Chiari malformation manifesting as extreme bradycardia, diaphragm impairment, and decreased coordination and was treated with a ventriculoperitoneal shunt. Exocrine pancreatic function was assessed by
Fecal elastase measurement and serum trypsin and amylase. Fecal elastase levels were normal in all patients who were able to provide stool specimens (Table 2). Serum trypsin levels were normal except for a mild elevation in patient 206 (who had normal fecal elastase). Serum amylase levels were normal for all patients (data not shown). All AHO patients participating in this study were screened for the 28 most common mutations in CFTR that account for approximately 85% of CF alleles. One patient (213) was heterozygous for the ∆F508 mutation and therefore excluded from any functional CFTR analysis. Finally, CFTR function was examined using pilocarpine-induced sweat chloride levels that reflect cholinergic activation of CFTR. All AHO patients had normal-for-age sweat chloride measurements except for patient 213 (Table 2). Of note, the three patients with CF-like responses in both nasal epithelium and sweat glands had normal pulmonary and pancreatic function.

Discussion

Gsaα-mediated signaling couples a large number of receptors with their target effectors via the production of cAMP. Ubiquitous expression, high degree of amino acid conservation across diverse species, and recent links to disease phenotypes illustrate the key support role Gsaα plays in many cellular functions. Yet direct assessment of the consequences of reduced Gsaα signaling in humans has been limited to hormonal and radiological studies in patients with mutations in GNAS and inference from normal human tissues and murine models. Although tissue-specific preferential expression and haploinsufficiency (28, 36) have been suggested, the mechanisms by which abnormalities in Gsaα signaling cause phenotypes such as Albright hereditary osteodystrophy are not known. The current study is the first to evaluate the in vivo effects of altered Gsaα signaling due to mutation in one GNAS gene on a downstream effector. In contrast to endocrine tissues, we found no evidence of preferential expression of Gsaα transcripts in epithelial cells. Signaling via Gsaα in AHO patients was reduced as documented by decreased maximal cAMP production. Reduced Gsaα signaling in the AHO patients was associated with decreased CFTR activation in vivo when compared with normal controls. Thus, mutation of one GNAS gene causes reduced Gsaα signaling in epithelial tissues of AHO patients and leads to significant decreases in the activation of downstream targets.

Tissue-specific preferential expression of Gsaα from the maternal allele of GNAS is postulated to account for the hormone resistance phenotype seen in some AHO patients. The observation that mice bearing heterozygous GNAS disruptions have significantly decreased mRNA and protein levels in renal cortex and thyroid as well as higher PTH and TSH levels in serum when the maternal allele is disrupted supports the concept of preferential expression (2, 3). Given that the hormone-responsive tissues involved in this phenotype are derived from all three primordial layers and Gsaα expression is biallelic in many tissues, this theory would have to involve a selective replacement or removal of the paternal imprint signal after gametogenesis and relatively late in embryogenesis. We did not find evidence of preferential expression of Gsaα in nasal epithelial tissue from 16 normal controls. Our results indicate that if reduction of paternally derived Gsaα transcripts accounts for the hormone resistance seen in a subset of AHO patients, then imprinting of the paternal GNAS gene has to occur late in embryonic development because both lung epithelium and thyroid are derived from embryonic endoderm.

The mechanism by which GNAS mutation causes bone and metabolic defects in AHO patients is largely unknown. Because expression of Gsaα is biallelic in bone, adipose, liver, and central nervous system tissue, haploinsufficiency has been suggested as a candidate mechanism. Chimeric mice consisting of wild-type and GNAS +/− cells did not show...
growth defects, but within the growth plate, the +/− chondrocytes underwent premature hyperplasia, compared with their wild-type counterparts (25). Thus, decreased Gsa expression was linked to a subtle downstream phenotype in mice. In our current study, we demonstrate decreased Gsa signaling in humans with an inactivating GNAS mutation and subsequent defects in a known downstream target. Although we did not quantify GNAS RNA expression directly, we found significant reductions in cAMP responses from AHO patients and therefore show a reduction in functional Gsa. Downstream CFTR activity was decreased in AHO patients in vitro, both in skin and respiratory epithelia, thus clearly demonstrating haploinsufficiency in humans.

We have shown that Gsa is central to the β-adrenergic-stimulated activation of CFTR in that decreases in Gsa signaling led to reduced cAMP generation and reduced CFTR activation in vitro as seen in the NPD and sweat rate responses. Sweat rates in AHO patients were notably similar to CF, implying that sweat production is a close proxy to activated CFTR function or that the sweat gland is more sensitive than respiratory epithelium to defects in CFTR activation. Durie and colleagues (37) have recently shown broad ranges of nasal potential differences in patients with mild CF. These responses are similar to what was seen in AHO patients. Yet AHO patients do not manifest the progressive obstructive lung disease or pancreatic insufficiency found in CF. Our data question the long-standing premise that β-adrenergic stimulation of CFTR is central to the pathophysiology of CF. Loss of basal CFTR function or other activated function may be more relevant to the pathogenesis of CF. Alternatively, AHO patients may have compensatory changes downstream of CFTR that mitigate lung and pancreatic disease severity, i.e. inflammation or host defense pathways. However, we cannot completely discount the role of the β-adrenergic pathway in the development of CF lung disease. The prevalence of lung dysfunction discernible by PFT was relatively high in AHO patients. Age, body habitus, and cognitive ability could not completely account for these abnormalities. β-Adrenergic receptor (ADRB2) variants have been associated with lung function in CF patients (38, 39). However, no association was seen between ADRB2 genotype and bronchodilator response, arguably the most direct in vivo measure of this pathway. We did not find an association between ADRB2 genotypes and cAMP generation in AHO patients (data not shown).

Our study demonstrates that GNAS mutations lead to haploinsufficiency of Gsa signaling in epithelial cells and reduced activation of CFTR. We speculate that haploinsufficiency may also contribute to the bone and metabolic defects seen in AHO patients, although identification of the appropriate downstream targets and access to these tissues currently preclude the execution of these studies. Although we found that AHO patients as a group have decreased cAMP generation and decreased CFTR activation, variability in both measures were apparent and some patients with low cAMP levels had normal CFTR activation.

Acknowledgments

The authors thank the participants and their referring physicians as well as Lois Brass-Ernst, R.N., for help with the NPDs and sweat rates.

Received February 6, 2007. Accepted July 18, 2007.

Address all correspondence and requests for reprints to: Stephanie C. Hsu, M.D., Ph.D., Johns Hopkins University, 723 North Broadway, Suite 551, Baltimore, Maryland 21205. E-mail: shsu86@jhmi.edu.

This work was supported by National Institutes of Health Grants T32 DK007751 (to S.C.H.), F32 DK067748 (to S.C.H.), K12 HD27799 (to S.C.H.), R01 DK 44003 (to G.C.), and R01 HL68927 (to G.R.C.); U.S. Food and Drug Administration Orphan Products Development Grant R01 FD-R-002568 (to E.L.G.-L.); Thrasher Research Foundation Grant 02818-8 (to E.L.G.-L.); and Johns Hopkins University General Clinical Research Center Grant M01 RR00052.
References


