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GATA3 haploinsufficiency does not block allergic sensitization or atopic disease

Monica G. Lawrence, MD¹, Jennifer W. Leiding, MD^{2,3,*}, Jonathan J. Lyons, MD^{4,*}, Amy P. Hsu, BS², Celeste C. Nelson, CRNP⁴, Nina Jones, RN⁵, Alan Fitzgerald, RN², Wade W. Chien, MD^{6,7}, Lisa Workman, BA¹, Thomas A. Platts-Mills, MD¹, Carmen Brewer, PhD⁶, Rachel I. Gafni, MD⁸, Kelly D. Stone, MD, PhD⁴, Joshua D. Milner, MD^{4,#}, and Steven M. Holland, MD^{2,#}

¹Asthma and Allergic Diseases Center, University of Virginia Health System, Charlottesville, Virginia ²Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases (NIAID), NIH, Bethesda, Maryland ³Division of Allergy, Immunology, and Rheumatology, Department of Pediatrics, University of South Florida, St. Petersburg, Florida ⁴Laboratory of Allergic Diseases, NIAID, NIH, Bethesda, Maryland ⁵Clinical Research Directorate/CMRP, Leidos Biomedical Research Inc., National Laboratory for Cancer Research, Frederick, Maryland ⁶Division of Intramural Research, National Institute on Deafness and Other Communication Disorders, NIH, Bethesda, Maryland ⁷Department of Otolaryngology - Head and Neck Surgery, Johns Hopkins School of Medicine, Baltimore, Maryland ⁸National Institute of Dental and Craniofacial Research, NIH, Bethesda, Maryland

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To the Editor

GATA3 is the major transcription factor that drives Th2 differentiation and inhibits T-BET driven Th1 differentiation from naïve CD4+ T cells in response to Th2 skewing conditions [1]. GATA3 is also expressed in the developing parathyroid glands, inner ear, and kidneys and is critical to vertebrate embryonic development [2]. *Gata3* homozygous knockout mice (*Gata3*^{-/-}) have a total block of T cell differentiation and die in utero [3, 4], while hemizygous conditional deletion of *Gata3* in CD4+ T cells (*Gata3*^{fl/+}) leads to impaired IL-4 and IgE production, Th1 cytokine production even in Th2 priming conditions, and

Address Correspondence to: Steven M. Holland, MD, 10 Center Drive, CRC B3-4141, MSC 1684, Bethesda MD 20892-1684, smh@nih.gov, 301-451-9019 (telephone), 301-480-4507 (fax).

*Authors contributed equally to this work

#Authors contributed equally to this work

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failure to control parasitic infection, suggesting a *Gata3* gene dosage effect on IL-4 mediated Th2 responses [5]. In humans, haploinsufficiency of GATA3 leads to a rare autosomal dominant condition characterized by variably penetrant hypoparathyroidism, sensorineural hearing loss (deafness) and renal anomalies (HDR syndrome) [6]. Previously published work in patients with HDR syndrome has demonstrated an absence of humoral or cellular immunodeficiency, with no clinical history of recurrent infections [1, 6, 7], but has suggested a modest impairment in Th2 responses *in vitro*. However, further investigation into the functional and clinical relevance of this impairment *in vivo* is lacking.

We therefore examined 4 patients from 3 families with HDR syndrome for their ability to mount a clinical allergic response as well as produce evidence of allergic sensitization on skin prick and serum IgE testing (please see the Methods section in this article's Online Repository at www.jacionline.org). Phenotypic variation was found within one family (patients 1 and 2) agreeing with previous reports of variable expressivity within families with HDR syndrome (Table E1) [7, 8]. Within our cohort three novel missense mutations (L284R, G289E, M294R) and 1 previously described single base pair deletion (c.708delC) were identified in *GATA3* [8]. None of the patients had a history of severe/recurrent infection or autoimmunity. There were no abnormalities in white blood cell counts or T lymphocyte subsets. Immunoglobulin production and response to vaccines were normal. Autoantibodies associated with rheumatologic disease were absent.

Patient 4 reported a history of significant atopic disease including allergic rhinitis, atopic dermatitis and food allergy to soybean and peanut consisting of immediate urticaria with ingestion (Table 1). This allergy developed in early childhood and persisted into adulthood. Skin prick testing of this patient to a panel of foods and environmental allergens was positive only to cockroach mix and soybean. All other skin prick tests were negative and there was no detectable allergen-specific IgE (sIgE); total serum IgE was not elevated (11.1 IU/ml, normal 0–90 IU/mL). Among the other three patients, despite a negative clinical history of atopic disease, two had evidence of sensitization to at least one aeroallergen identified by skin prick test. None of the patients had an elevated total serum IgE and none had detectable sIgE to any allergens tested.

We next sought to characterize the cytokine production by circulating peripheral blood T lymphocytes from these patients. Analysis of cytokine production by CD3⁺CD4⁺CD27⁺CD45RO⁺ effector memory T cells following stimulation with PMA/ionomycin demonstrated a trend towards an increased frequency of IFN- γ producing cells and a trend towards a decreased frequency of IL-4 and IL-13 producing cells in patients (*GATA3*^{+/-}) compared to healthy controls (*GATA3*^{wt}), although there was wide variability and this did not reach statistical significance (Fig 1). As previous reports have suggested a Th1 bias with a concomitant impairment in Th2 cytokine production[1], we next sought to determine if there was a defect in Th2 differentiation. Naïve CD4⁺ lymphocytes were subject to Th0 or Th2 skewing conditions. Although induction of *GATA3*⁺ cells was seen in both groups under Th2 skewing conditions, the fold-induction in *GATA3* was greater in healthy controls (*GATA3*^{wt}) than in patients (*GATA3*^{+/-}) (Fig 2a,b), suggesting that T lymphocytes from *GATA3* haploinsufficient patients were more resistant to Th2 priming than controls.

It is now well established that GATA3 is the major transcription factor that drives Th2 differentiation. Loss of one functional *GATA3* allele (*GATA3* haploinsufficiency) in humans leads to significant embryonic consequences and is the cause of the HDR syndrome characterized by hypoparathyroidism, sensorineural hearing loss, and renal anomalies [6]. We report four adult patients with HDR syndrome, two with novel missense mutations. Prior work has demonstrated *in vitro* Th1 skewing with a modest concomitant impairment in the Th2 compartment in patients with HDR syndrome [1]. We confirm a modest Th1 bias and Th2 defect *in vitro*. However, the potential clinical consequences of this Th1/Th2 imbalance have not previously been investigated. One patient in our cohort reported significant atopic disease including allergic rhinitis, food allergy and eczema, suggesting that the *in vitro* Th1 skewing does not inhibit the ability to produce a Th2-mediated allergic response *in vivo*. Interestingly, we noted discordance between skin prick and serum allergy testing results. None of our patients had an elevated serum total IgE or detectable specific IgE to a panel of common environmental and food allergens, while all four patients had sensitization to at least one allergen as identified by skin prick test. Although discordance between serum allergen specific IgE and skin prick test results has been described in large population studies [9], it is possible that a reduction in GATA3-driven IL-4 production leads to impairment of detectable serum antigen specific IgE while preserving the capacity for skin prick and clinical sensitization. This could be explored using larger cohorts of patients with HDR syndrome, as well as in atopic controls in whom discordance between skin and serum testing has been observed. Furthermore, modest Th1 skewing appears to be held in check without evidence of clinical autoimmunity. These data suggest that while the impact of GATA3 during embryogenesis on certain targets is highly dose-dependent, CD4+ T lymphocyte development and function and Th2 responses are largely insensitive to GATA3 haploinsufficiency and allow clinical allergic disease.

METHODS

Human subjects and clinical studies

All studies involving human subjects were performed in accordance with National Institute of Allergy and Infectious Diseases Institutional Review Board–approved protocols 10-I-0148 and 00-I-0159 and the National Institute of Dental and Craniofacial Institutional Review Board–approved protocol 01-D-0184, as well as the guidelines in the 1964 Declaration of Helsinki and its later amendments. All patients gave informed consent prior to their inclusion in the study. Allergic, infectious, and autoimmune histories, immunologic phenotyping, allergen skin prick testing (SPT), serum total and allergen specific IgE level, peripheral absolute eosinophil counts, and routine biochemistries were measured in four patients with a clinical history of HDR syndrome. A renal ultrasound and assessment of the auditory and vestibular systems were also performed. In each patient, a mutation in GATA3 was identified by Sanger sequencing. Controls are age- and sex-matched healthy volunteers.

Skin prick testing (SPT)

SPT to a panel of 7 food [cashew nut, egg white, cow's milk, peanut, shrimp, soybean, wheat] and 13 aeroallergen [*Alternaria alternata* (tenuis), *Aspergillus fumigatus*, birch mix (black/sweet, red/river, white), black walnut tree, cat hair, cockroach mix (German,

American), *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, dog dander, mouse epithelium, oak mix (black, red, white), ragweed mix (giant, short/common), timothy grass] extracts (United States Army Centralized Allergen Extract Laboratory, Bethesda MD) was performed on the forearm with a Greer Pick (Greer Laboratories, Lenoir NC). Positive histamine and negative diluent controls were used. Reactions were recorded at 15 minutes. A wheal of 3 mm greater than the negative control was considered positive.

ImmunoCAP assay

Sera were analyzed for allergen-specific IgE (sIgE) antibodies to a panel of 8 food and 16 aero-allergens; allergens matched those used for SPT with the addition of walnut, cat epithelium, *Cladosporium herbarum*, and lambs quarter. Analysis was performed by a commercial laboratory (Mayo Medical Laboratories, Rochester MN) or by using the Phadia ImmunoCAP System (Phadia AB, Uppsala, Sweden) as detailed by the manufacturer. Values of 0.35 kU/L or more were considered positive.

T cell differentiation

PBMCs were isolated by Ficoll-Hypaque density centrifugation and resuspended in R10 medium (RPMI 1640 supplemented with 100 U/mL of penicillin, 100 µg/mL of streptomycin, 29.2 µg/mL of l-glutamine (all GIBCO, Carlsbad CA) and 10% heat-inactivated FCS (Gemini Bio-Products, Sacramento CA)). Naïve CD4⁺ T cells were isolated from PBMCs using negative selection MACS microbeads (Miltenyi Biotec, San Diego CA) as per manufacturer's protocol. Purity was >90% in all samples run. Cells were stimulated in 96 well round-bottom plates at 50,000 cells per well that had been coated with 1 µg/ml anti-CD3 (OKT3 clone). Th2 cultures were performed in X-VIVO 15 medium (Lonza, Basel, Switzerland) in the presence of IL-4 (12.5ng/ml), anti-IFN-γ (10µg/ml), anti-IL-10 (10µg/ml), anti-CD28 (1µg/ml) and IL-2 (10 IU/ml) for 5.5 days. Th0 cell cultures were performed without skewing cytokines.

Flow cytometry

For transcription factor analysis following in vitro differentiation, cells were washed and surface stained with Live/Dead Fixable Aqua viability dye (Invitrogen, Carlsbad CA), fixed and permeabilized with the Foxp3 staining kit and stained for CD4 FITC, Gata3 AF-647 (both BD Biosciences, San Jose CA), and Tbet PE (eBioscience, San Diego CA).

For *ex vivo* CD4⁺ memory T cell cytokine analysis, PBMCs were examined after stimulation for 6 hours with 20 ng/mL of phorbol 12-myristate 13-acetate (PMA) and 1 µM of ionomycin in the presence of 10 µg/mL of brefeldin-A for the last 4 hours. Cells were surface stained with Live/Dead Fixable Aqua viability dye, CD3 AF-700, CD4 PE-Cy7, CD8 APC-H7 (all BD Biosciences, San Jose CA), CD27 PE-Cy5 and CD45RO Texas Red PE (Beckman Coulter, Brea CA), fixed with 4% paraformaldehyde, permeabilized with a saponin-based buffer, and stained intracellularly for IFN-γ FITC, IL-17 PE, and IL-22 APC or IL-2 FITC, IL-4 PE and IL-13 APC. Events were collected on an LSRFortessa (BD Biosciences) and analyzed with FlowJo 9.7.5 (Treestar, Ashland OR). All plots are gated on live, singlet, CD3⁺CD4⁺ lymphocytes.

Statistical analysis

Results are reported as means $\pm$ SEMs, unless otherwise stated. Differences between groups were assessed using the paired t-test or the Mann-Whitney test (Graph Pad Prism; Graph Pad Software, San Diego CA). The statistical significance level adopted was a P value of less than 0.05.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Skapenko A, Leipe J, Niesner U, Devriendt K, Beetz R, Radbruch A, et al. GATA-3 in human T cell helper type 2 development. *The Journal of experimental medicine*. 2004; 199(3):423–8.10.1084/jem.20031323 [PubMed: 14757746]
2. Debacker C, Catala M, Labastie MC. Embryonic expression of the human GATA-3 gene. *Mechanisms of development*. 1999; 85(1–2):183–7. [PubMed: 10415360]
3. Pandolfi PP, Roth ME, Karis A, Leonard MW, Dzierzak E, Grosveld FG, et al. Targeted disruption of the GATA3 gene causes severe abnormalities in the nervous system and in fetal liver haematopoiesis. *Nat Genet*. 1995; 11(1):40–4.10.1038/ng0995-40 [PubMed: 7550312]
4. Ting CN, Olson MC, Barton KP, Leiden JM. Transcription factor GATA-3 is required for development of the T-cell lineage. *Nature*. 1996; 384(6608):474–8.10.1038/384474a0 [PubMed: 8945476]
5. Zhu J, Min B, Hu-Li J, Watson CJ, Grinberg A, Wang Q, et al. Conditional deletion of Gata3 shows its essential function in T(H)1-T(H)2 responses. *Nature immunology*. 2004; 5(11):1157–65.10.1038/ni1128 [PubMed: 15475959]
6. Van Esch H, Groenen P, Nesbit MA, Schuffenhauer S, Lichtner P, Vanderlinden G, et al. GATA3 haploinsufficiency causes human HDR syndrome. *Nature*. 2000; 406(6794):419–22.10.1038/35019088 [PubMed: 10935639]
7. Muroya K, Hasegawa T, Ito Y, Nagai T, Isotani H, Iwata Y, et al. GATA3 abnormalities and the phenotypic spectrum of HDR syndrome. *Journal of medical genetics*. 2001; 38(6):374–80. [PubMed: 11389161]
8. Ali A, Christie PT, Grigorieva IV, Harding B, Van Esch H, Ahmed SF, et al. Functional characterization of GATA3 mutations causing the hypoparathyroidism-deafness-renal (HDR) dysplasia syndrome: insight into mechanisms of DNA binding by the GATA3 transcription factor. *Human molecular genetics*. 2007; 16(3):265–75.10.1093/hmg/ddl454 [PubMed: 17210674]
9. de Vos G. Skin testing versus serum-specific IgE testing: which is better for diagnosing aeroallergen sensitization and predicting clinical allergy? *Current allergy and asthma reports*. 2014; 14(5): 430.10.1007/s11882-014-0430-z [PubMed: 24633614]

Fig. 1.

Results of a six-hour stimulation of PBMCs with PMA and ionomycin in the presence of brefeldin A, followed by fixation, permeabilization and staining for intracellular cytokines. Data shown represent mean of 3 independent experiments for GATA3^{+/-} patients; included for comparison are age and sex matched healthy controls.

Fig. 2. CD4⁺ naïve T cells were subject to Th0 (anti-CD3/CD28 and IL-2) or Th2 (anti-CD3/CD28, IL-2, IL-4, anti-IFN- γ , anti-IL-10) skewing conditions prior to staining for expression of TBET and GATA3. n=3–5 independent experiments. (a) Geometric mean fluorescent intensity (MFI) of GATA3 under Th0 and Th2 conditions (representative data). (b) Fold increase of GATA3 MFI under Th2 conditions compared to Th0 conditions.

Table 1

Atopic evaluation of GATA3 haploinsufficient subjects

Clinical History	Patient 1	Patient 2	Patient 3	Patient 4
Allergic Rhinitis	None	None	None	Seasonal allergic rhinitis managed with daily oral antihistamines and intranasal corticosteroids
Asthma	None	None	None	None
Atopic Dermatitis	None	None	None	Yes, as a child
Food Allergy	None	None	None	Soy and peanuts - urticaria
Chronic Urticaria/Angioedema	None	None	None	None
Anaphylaxis	None	None	None	None

Skin prick testing wheal x flare, in mm)	Patient 1	Patient 2	Patient 3	Patient 4
Histamine (positive control)	6x30	6x5	5x8.5	5.5x27.5
Morphine 10mg/ml (positive control)	4.5x12	3x3	3.5x3.5	4.5x12.5
Saline (negative control)	0x0	0x0	0x0	0x0
Aeroallergens (13 tested)	<i>D. pteronyssinus</i> 4x13	None	<i>D. pteronyssinus</i> 3x3 Mouse epithelium 3x3 Oak mix 3x3	Cockroach mix 3x3
Food allergens (7 tested)	None	None	None	Soybean 3x3

Blood Testing	Patient 1	Patient 2	Patient 3	Patient 4
Absolute eosinophil count <i>Normal range 40–360 cells/μl</i>	40 cells/μL	90 cells/μL	170 cells/μL	80 cells/μL
Serum total IgE <i>Normal range 0–90 IU/ml</i>	5.6 IU/ml	21.9 IU/ml	72.3 IU/ml	11.1 IU/ml
Aeroallergen sIgE (14 tested)	All <0.35 kU/ml	All <0.35 kU/ml	All <0.35 kU/ml	All <0.35 kU/ml
Food allergen sIgE (8 tested)	All <0.35 kU/ml	All <0.35 kU/ml	All <0.35 kU/ml	All <0.35 kU/ml