

a lower molecular weight band of approximately 437 bp was identified in cDNA from the patient's BLCLs. Sanger sequencing demonstrated abnormal splicing in the patient's cDNA amplicon, resulting in deletion of exon 13 and in-frame splicing of exons 12 and 14 (Fig 1, C). Immunoblotting of lysates from BLCLs demonstrated abrogation of STAT2 protein expression in our patient without expression of any truncated protein products (Fig 1, D).

STAT2 deficiency in human subjects and mice is characterized by defective type I interferon signaling.^{1,2,7} The infection and subsequent transformation of B cells with EBV activates STAT2 and upregulates expression of interferon-stimulated genes, including *IFIT1*, *ISG15*, and *IRF7*, which are further increased after exposure to type I interferons.^{8,9} Expression of *IFIT1*, *ISG15*, and *IRF7* was severely decreased in the patient's BLCLs compared with those from control subjects (time 0 in Fig 1, E). Furthermore, the patient's BLCLs did not upregulate *IFIT1*, *ISG15*, and *IRF7* after IFN- α stimulation (Fig 1, E), indicating a lack of STAT2-driven signaling in response to type I interferons.

Mouse models of STAT2 deficiency have demonstrated defective responses to viral infections both *in vitro* and *in vivo*.¹ Human STAT2 deficiency has been associated with development of recurrent and severe viral illnesses. After measles, mumps, and rubella vaccination, 1 patient had disseminated vaccine-strain measles virus,¹ another patient had meningitis with vaccine-strain mumps virus,⁷ and 4 patients had post-measles, mumps, and rubella vaccine febrile syndromes with negative viral study results.^{1,2,7}

This is the first report of HLH in association with STAT2 deficiency. We identified a novel STAT2 variant in our patient, resulting in abrogation of protein expression and type I interferon response. This report, as well as previously published reports of patients with defects in *STAT1* and *IFNGR2*, demonstrate that secondary HLH can occur with defects in either type I or type II interferon signaling.^{4,5} The rapid improvement of our patient after high-dose IVIG suggests the utility of this intervention in treating secondary HLH triggered by infection. The beneficial effect of IVIG might be ascribed to passive immunization and possibly to its potential anti-inflammatory effect. Our report and other literature² suggests patients with STAT2 deficiency might benefit from monthly immunoglobulin replacement during childhood, particularly if they have received live vaccines before their molecular diagnosis, until their adaptive immune systems mature.

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GATA-2-deficient mast cells limit IgE-mediated immediate hypersensitivity reactions in human subjects



To the Editor:

GATA proteins are lineage-restricted transcription factors that regulate tissue-specific patterns of gene expression, principally during development. GATA-2 is expressed in hematopoietic stem cells and multilineage progenitors, where it plays essential roles in their maintenance and proliferation.¹ Indeed, *Gata2*-null mice succumb at embryonic day 10.5 with severe anemia caused by bone marrow failure.² GATA-2 also appears to regulate early mast cell gene expression^{3,4} and has been shown to positively regulate mast cell identity in model systems.^{5-9,E1-E3}

GATA-2 deficiency is a protean disorder in human subjects with markedly variable clinical expressivity. Relevant to the mast cell compartment, many GATA-2-deficient subjects have myeloid lineage abnormalities, including marrow hypocellularity, cytopenias, and myeloid neoplasms.^{4,E4-E6} Given that the data available on the effects of GATA-2 disruption in mast cells are limited to nonhuman or *in vitro* studies, we examined the effects of heterozygous loss-of-function *GATA2* mutations on clinical allergy, cultured primary mast cell growth, morphology, and activation in patients with *GATA2* deficiency.

Retrospective investigation revealed the overall prevalence of documented clinical symptoms consistent with IgE-mediated immediate hypersensitivity to foods, environmental allergens, medications, and/or stinging insects to be only 21% (6/28) in GATA-2-deficient subjects, whereas 49% (454/927) of all remaining subjects on the same clinical protocol had documentation of immediate hypersensitivity reactions ($P = .004$; Fig 1, A) (see the Methods section in this article's Online Repository at www.jacionline.org). The prevalence of type I allergy in this control cohort, which includes healthy

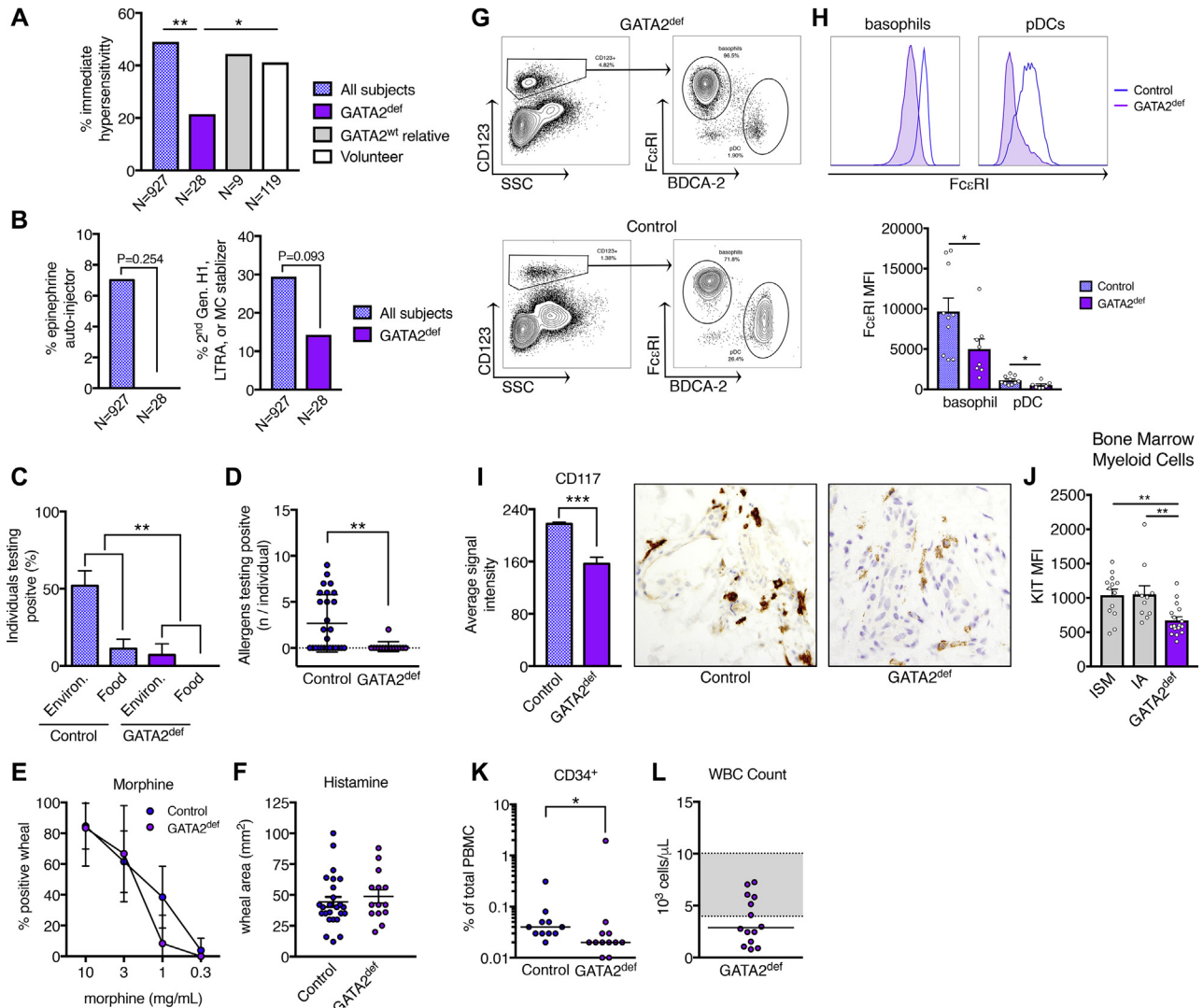


FIG 1. Reduced allergen-specific skin reactivity and clinical allergy in GATA-2-deficient patients are associated with reduced KIT and FcεRI expression. **A**, Prevalence of IgE-mediated reactions in GATA-2-deficient subjects (*GATA2*^{def}), unaffected family members (*GATA2*^{wt}), and remaining subjects (*All subjects*), as well as a second healthy volunteer cohort (*Volunteer*). **B**, Epinephrine autoinjectors (*left*) and second-generation antihistamines (*2nd Gen. H1*), leukotriene receptor antagonists (*LTRA*), or mast cell (*MC*) stabilizer prescriptions (*right*). **C** and **D**, Number of allergens (Fig 1, **C**) and percentage of subjects (Fig 1, **D**) with positive SPT responses to 11 environmental and 8 food allergens. **E** and **F**, Morphine (Fig 1, **E**) and histamine (Fig 1, **F**) SPT responses in control subjects (*n* = 26) and subjects with GATA-2 deficiency (*n* = 14 and 12, respectively). **G** and **H**, Gating strategy (Fig 1, **G**) and representative histograms (*top*) and combined extracellular staining of FcεRI (*bottom*) from GATA-2-deficient subjects (*n* = 8) and healthy volunteers (control; *n* = 10; Fig 1, **H**). **I**, Staining intensity of KIT (CD117) on skin mast cells quantified (*left*) and representative immunohistochemistry images (*right*). **J**, KIT mean fluorescent intensity (*MFI*) of myeloid cells from bone marrow aspirates of subjects with GATA-2 deficiency (*n* = 17), idiopathic anaphylaxis (*IA*; *n* = 11), or indolent systemic mastocytosis (*ISM*; *n* = 13). **K** and **L**, Total WBCs (adult normal reference ranges are indicated in gray; Fig 1, **L**) and CD34⁺ progenitor frequency (Fig 1, **K**). Fisher exact, Mann-Whitney, and unpaired 2-tailed *t* tests: **P* < .05, ***P* < .005, and ****P* < .0001.

volunteers, subjects being screened for immunodeficiency diseases, and unaffected family members, was comparable with that in relatives of GATA-2-deficient subjects confirmed to be GATA-2 wild-type (*GATA2*^{wt}; 44% [4/9]), well-phenotyped healthy volunteers (41% [41/119]), and US national prevalence of sensitization and combined estimates for IgE-mediated allergic diseases.^{E7-E9} Consistent with this finding, none of the GATA-2-deficient subjects had been prescribed epinephrine

autoinjectors compared with 7% (65/927) of all other subjects, and only 4 (14%) had been prescribed second-generation antihistamines, leukotriene modifiers, or mast cell stabilizers compared with 29% (273/927) of all other subjects (Fig 1, **B**, and see Table E1 in this article's Online Repository at www.jacionline.org).

Fourteen consecutive GATA-2-deficient subjects subsequently underwent prospective clinical evaluation (see Table E2 in this

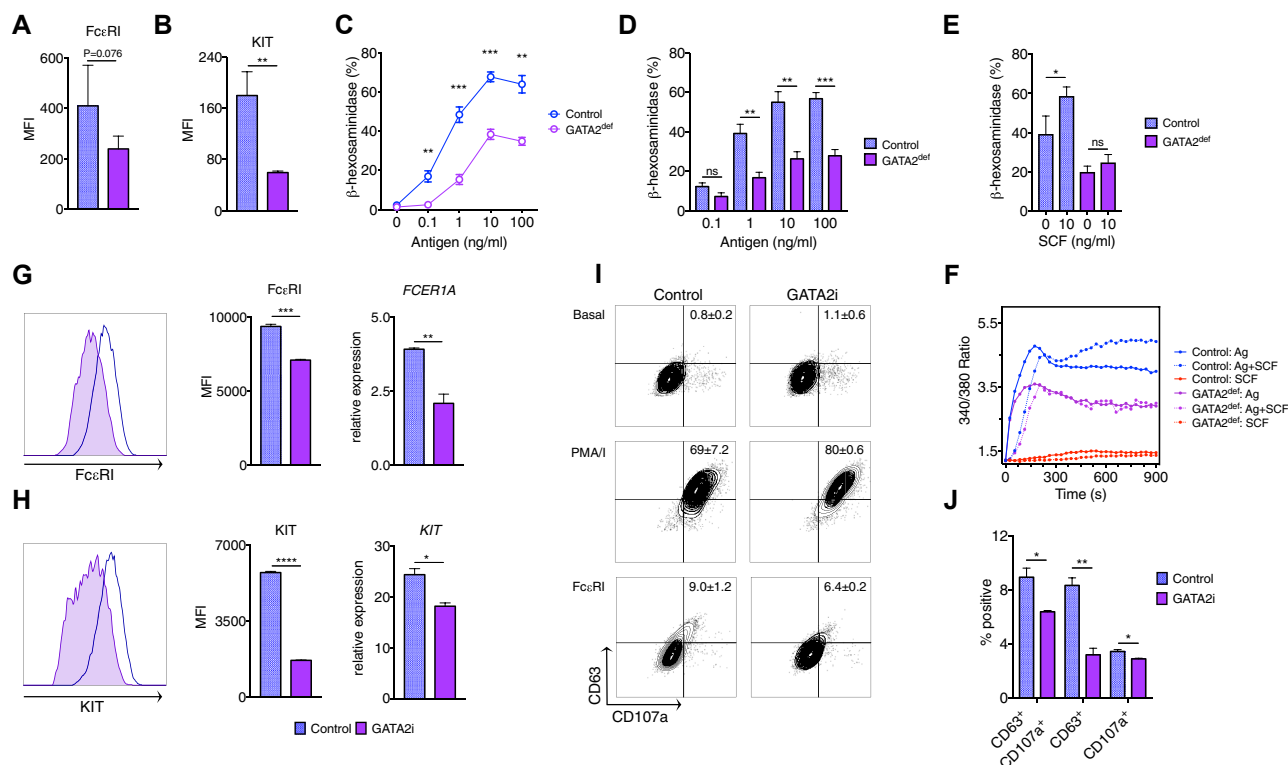


FIG 2. GATA-2 deficiency and inhibition are associated with reduced FcεRI and KIT expression to limit IgE-mediated degranulation in human mast cells. **A** and **B**, FcεRI (Fig 2, **A**) and KIT (Fig 2, **B**) surface expression in primary cultured mast cells from GATA-2-deficient subjects and paired control subjects from 3 independent cultures. *MFI*, Mean fluorescent intensity. **C** and **D**, Representative (Fig 2, **C**) and combined (Fig 2, **D**) data from 4 independent experiments demonstrating antigen-mediated degranulation as measured based on β-hexosaminidase release in primary cultured mast cells. **E**, β-Hexosaminidase release in response to antigen (1 ng/mL) in the presence or absence of SCF. **F**, Calcium flux after antigen (Ag)-mediated IgE cross-linking of primary cultured mast cells in the presence or absence of SCF; representative data are from 2 independent experiments. **G** and **H**, Representative histograms (left) and combined surface expression (middle) of FcεRI (Fig 2, **G**) and KIT (Fig 2, **H**) after treatment with the GATA-2 inhibitor K-7174 (GATA2i) or dimethyl sulfoxide diluent (Control), and relative *FCER1A* and *KIT* gene expression (right) under the same conditions. **I** and **J**, Representative contour plots of CD63 and CD107a expression after phorbol 12-myristate 13-acetate/ionomycin or biotinylated IgE cross-linking by streptavidin (FcεRI; Fig 2, **I**) and combined data (Fig 2, **J**) resulting from FcεRI cross-linking. All data are from 3 or more independent cultures. Mann-Whitney and unpaired 2-tailed *t* tests: **P* < .05, ***P* < .005, and ****P* < .0001, and *****P* < .0001.

article's Online Repository at www.jacionline.org). Only 2 subjects reported a history of seasonal allergic rhinitis and no other allergic disease, whereas 9 (64%) of 14 families had 1 or more GATA-2^{wt} family members with a physician-diagnosed IgE-mediated allergic disease. All GATA-2-deficient subjects and 27 unselected volunteers (control) underwent skin prick tests (SPTs) to a panel of common environmental allergens endemic to a region they frequent or where they lived as well as common food allergens (see Table E3 in this article's Online Repository at www.jacionline.org). Control subjects demonstrated a greater rate of sensitization (*P* = .0039; Fig 1, **C**), and a greater number were polysensitized relative to GATA-2-deficient subjects (14 vs 1, *P* = .004; Fig 1, **D**). SPT responses in GATA-2-deficient subjects were discordant with specific IgE levels in serum, where despite low total IgE levels (median IgE, 5.9 kU/L), 4 subjects had detectable antigen-specific IgE, 3 of whom were polysensitized to 3 or 4 allergens (see Fig E1 and Table E2 in this article's Online Repository at www.jacionline.org); sensitization correlated with clinical symptoms in 1 subject.

Because *in vitro* data have demonstrated reduced protein kinase Cβ levels in RBL-2H3 cells associated with GATA-2 inhibition,^{E10} an effect that should limit non-IgE-mediated degranulation,^{E11} SPT to titrated morphine was also performed in GATA-2-deficient subjects (*n* = 12) and control subjects (*n* = 26); no significant difference was detected in wheal development (Fig 1, **E**). Furthermore, GATA-2-deficient subjects did not have impaired responses to the mast cell mediator histamine, as quantified by wheal area (Fig 1, **F**), suggesting the reduced SPT responses and clinical allergy observed were both mast cell intrinsic and antigen dependent.

We next set out to identify mechanisms leading to impairment of antigen-dependent degranulation in GATA-2-deficient mast cells and identified KIT and FcεRI as candidates; these molecules both contribute to mast cell activation and survival in human subjects^{E12} and have been shown to depend on intact GATA-2 activity in a number of systems.^{3,4,6-9,E1,E10,E13} FcεRI expression was significantly reduced in basophils (*P* < .05) and plasmacytoid dendritic cells (*P* < .05) from GATA-2-deficient subjects *ex vivo*

(Fig 1, G and H), the latter of which were significantly reduced in number, as previously reported.^{E14} KIT expression was likewise reduced in GATA-2-deficient mast cells in skin biopsy specimens (Fig 1, I, and see Fig E2 in this article's Online Repository at www.jacionline.org), whereas mast cell numbers were paradoxically comparable with those of control subjects (see Fig E3 in this article's Online Repository at www.jacionline.org).

Although bone marrow mast cells identified in aspirates as CD45⁺KIT⁺CD34[−]SSC^{high} cells from patients with GATA-2 deficiency had comparable KIT expression when compared with those from control subjects with disease (see Fig E4, A, in this article's Online Repository at www.jacionline.org), these cells were actually increased ($P = .01$) in number compared with those in subjects with idiopathic anaphylaxis and comparable in number to subjects with indolent systemic mastocytosis (see Fig E4, B). Like indolent systemic mastocytosis, increased bone marrow mast cell counts were associated with increased basal serum tryptase (BST) levels (>11.4 ng/mL) in 3 of 11 subjects (see Fig E4, C). However, examining the remaining KIT⁺ myeloid cells in bone marrow, expression was found to be reduced (Fig 1, J). Likewise, significantly fewer CD34⁺ progenitor cells, which require KIT for survival,^{E15} were identified in the periphery of GATA-2-deficient patients after adjusting for leukopenia (Fig 1, K and L).

FcεRI and KIT expression were reduced in successfully cultured GATA-2-deficient mast cells (Fig 2, A and B). Although the majority of CD34⁺ cultures hypoproliferated, yielding too few mast cells for functional evaluation (see Fig E5, A and B, in this article's Online Repository at www.jacionline.org), 4 cultures exhibited transformation or expansion of previously transformed clones *in vitro*, despite completely losing KIT expression, wherein cultured cells displayed marked atypia and exponential growth in the absence of exogenous cytokine (see Fig E5, B-D). The positive correlation of GATA2 expression with KIT and FCER1A expression was validated in publicly available myeloid blast transcriptomic RNA sequencing data, as well as in GATA2^{wt} cultured mast cells, purified basophils, and mast cell lines (see Fig E6 in this article's Online Repository at www.jacionline.org).

Successfully cultured primary mast cells (from 5 GATA-2-deficient subjects and 6 paired healthy volunteers) were sensitized with biotinylated IgE, and FcεRI was cross-linked using streptavidin. Mast cell degranulation, as measured by β-hexosaminidase release, was reduced relative to control values (Fig 2, C and D). Stem cell factor (SCF) addition enhanced IgE-mediated mast cell degranulation in control subjects; however, it did not significantly alter GATA-2-deficient mast cell degranulation (Fig 2, E). Intracellular Ca²⁺ flux was likewise impaired in GATA-2-deficient mast cells in response to IgE cross-linking, as well as after the addition of SCF (Fig 2, F). These data are consistent with functional impairment in degranulation of primary GATA-2-deficient mast cells in response to both IgE and SCF, which was associated with the reduced FcεRI and KIT expression we have identified.

To test whether a short-term treatment could be used to block GATA-2 signaling and provide proof of concept for targeting this pathway as a potential therapy, the LAD2 human mast cell line was cultured in the presence of the small-molecule GATA-2 inhibitor K-7174 (GATA2i). We observed a dose- and time-dependent reduction in surface expression of FcεRI and KIT (Fig 2, G and H, and see Fig E7 in this article's Online

Repository at www.jacionline.org) and confirmed this was a target-specific effect not caused via proteasome inhibition (see Fig E8 in this article's Online Repository at www.jacionline.org).^{E16} Reduced KIT and FcεRI expression was associated with a significant reduction in IgE-dependent degranulation, as measured based on surface CD63 and CD107a (lysosomal-associated membrane protein 1) expression (Fig 2, I), but no significant differences were observed after IgE-independent degranulation achieved with phorbol 12-myristate 13-acetate/ionomycin treatment (Fig 2, J).

In summary, GATA-2-deficient subjects have evidence of impaired IgE-mediated mast cell activation and reduced IgE-mediated clinical allergic disease. These findings are antigen dependent and associated with reduced KIT and FcεRI expression and are not attributable to a numeric problem in mast cells or an impaired response to the mast cell mediator histamine.

Although our sample size is limited in patients with this rare disease, conserved numbers of skin and bone marrow mast cells identified in subjects with GATA-2 deficiency exist in stark contrast to the reduced numbers of CD34⁺ and total leukocytes^{E17} and despite diminished expression of KIT in skin, the key receptor for mast cell proliferation and homeostasis. How mast cells are able to overcome this impediment is unclear and remains a topic of interest. However, these data suggest that additional factors present *in vivo* might support mast cell differentiation and/or survival and compensate for impaired FcεRI- and KIT-dependent signaling.

In addition to the mast cell defects we have identified, antigen-specific and total IgE levels in sera from GATA-2-deficient subjects were low. B-cell lymphopenia and hypogammaglobulinemia have been reported in subjects with GATA-2 deficiency, and functional B-cell abnormalities can exist.^{E17,E18} Reduced numbers of antigen-presenting cells, most notably dendritic cells, have also been modeled and observed in the setting of GATA-2 deficiency and could contribute to impaired adaptive T_H2 responses.^{4,E5,E19} Although considerably challenging because of the degree of B-cell and dendritic cell cytopenia in these patients, careful future study of these compartments might provide additional insight into the potential effects of GATA-2 deficiency on allergic sensitization in human subjects.

Finally, new therapeutic targets are needed for the treatment of allergic diseases, and small molecules are of particular interest based upon considerations of cost and ease of administration. Short-term inhibition of GATA-2 with a small molecule successfully targeted both FcεRI and KIT expression, thereby limiting IgE-mediated mast cell activation *in vitro*. These patients provide proof of concept for targeting this pathway in human subjects provided appropriate delivery systems can be developed or untoward systemic effects can be overcome through rational drug design.^{E20,E21}

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Murine neonatal skin mast cells are phenotypically immature and minimally sensitized with transplacentally transferred IgE



To the Editor:

IgE binds to mast cells (MCs) through the high-affinity IgE receptor (FcεRI) on their surfaces and mediates type I hypersensitivity by inducing degranulation on recognition of its cognate antigen. It is generally considered that IgE does not pass through the placenta, unlike IgG, which is transplacentally transferred from mothers to fetuses through the neonatal Fc receptors (FcRn).^{1,2} However, subtle amounts of IgE are detected in umbilical cord blood in human subjects,³ suggesting the possibility of transplacental transfer of IgE.

IgE in umbilical cord blood is detected mostly in the form of immune complexes (ICs) with anti-IgE IgG autoantibodies,⁴ and ICs can bind to FcRn; therefore we hypothesized that transplacental IgE transfer can occur in the form of ICs. In fact, an *in vitro* transwell assay using a kidney cell line transfected with human FcRn showed transcytosis of IgE in the form of ICs.⁴ However, there has been no study to show transplacental IgE transfer *in vivo*. Furthermore, it is completely unknown whether transplacentally transferred IgE can distribute to peripheral tissues, such as the skin, and exert pathogenic functions in neonates. Here we aimed to elucidate (1) whether maternal IgE is transplacentally transferred in the form of ICs *in vivo* and (2) whether the transferred IgE distributes to the peripheral tissues in neonates and induces degranulation of MCs, focusing on the skin.

First, to investigate whether maternal IgE passes through the placenta, we injected anti-2,4,6-trinitrophenyl (TNP) IgEa, an allotype of murine IgE that does not exist in C57BL/6 mice, into pregnant C57BL/6 mice intravenously in either the monomeric form or the IC form on pregnancy day 17. Twenty-four hours later, we analyzed serum levels of anti-TNP IgEa in fetuses using an ELISA (Fig 1, A). In the mothers' sera, 24 hours after administration, levels of IC IgEa were substantially lower than those of the monomeric IgEa (see Fig E1 in this article's Online Repository at www.jacionline.org), but in fetuses, subtle amounts of IgEa were detected only in fetuses whose mothers were injected with ICs (Fig 1, B). These results indicate that maternal IgE passes through the placenta mainly in the form of ICs.

Next, we examined whether circulating IgE in the form of ICs can be distributed to the skin and bind to dermal MCs in neonates. First, we intraperitoneally administered anti-TNP IgEa in the form of ICs to postnatal day 0 (P0) mice. On the next day, we checked for the existence of IgE and anti-IgE IgG (goat IgG) on MCs in the skin by using flow cytometry. We detected surface binding of IgE and anti-IgE IgG (goat IgG) on MCs, but only approximately 7% of MCs were positive for IgE (and anti-IgE IgG; Fig 1, C). Consistent with this result,

METHODS

Study subjects

Patients, family members, and healthy volunteers provided informed consent on National Institute of Allergy and Infectious Diseases Institutional Review Board–approved research protocols designed to study mastocytosis or genetic diseases affecting the immune system (NCT00044122, NCT00001756, NCT01164241, NCT00852943, NCT00404560, NCT01905826, and NCT00806364).

Retrospective analysis of medical records was performed using the Biomedical Translational Research Information System. All medical records available in the National Institutes of Health Clinical Center medical records systems were queried for subjects on NCT00404560. All reactions were reviewed and classified as (1) consistent with an IgE-mediated immediate hypersensitivity reaction or (2) having no history of a reaction consistent with IgE-mediated allergy. Reactions in group 1 included immediate hypersensitivity reactions to foods, medications, and environmental allergens (seasonal rhinoconjunctivitis or perennial rhinoconjunctivitis with documentation of perennial allergen sensitization [eg, dust mite]) and systemic venom reactions. When the only reported symptom was rash, the eliciting agent was considered in assignment. Urticarial rashes caused by drugs, such as β -lactam antibiotics or sulfonamide medications, were assigned to group 1, whereas reactions to drugs associated with skin reactions commonly caused by non-IgE-mediated mechanisms, including corticosteroids, narcotics, and vancomycin, were assigned to group 2. Radiocontrast-mediated reactions, reports of iodine allergy, isolated pruritus, isolated gastrointestinal symptoms, or other reactions to medications not consistent with IgE-mediated allergy (eg, blistering rashes) were all assigned to group 2.

By using Biomedical Translational Research Information System, all prescribed medications for treatment of immediate hypersensitivity reactions were also queried (see Table E1 for the complete list). Because first-generation antihistamines, in particular diphenhydramine, were frequently prescribed for reasons unrelated to clinical allergic disease, only second-generation antihistamines, leukotriene modifiers, and mast cell stabilizers are reported as “allergy medications” (Table E1 provides the complete list of medications queried).

For prospective patient evaluation, 14 consecutive GATA-2–deficient patients underwent clinical evaluation. Comprehensive allergy histories, physical examinations, and reviews of outside records were performed at the Clinical Center of the National Institutes of Health. SPTs to a panel of common environmental ($n = 12$) and food ($n = 8$) allergens was performed by using histamine as a positive control (Table E3). Twelve GATA-2–deficient subjects also underwent SPTs with morphine. Total and allergen-specific serum IgE levels to the same allergens were also quantified by using ImmunoCAP (Phadia, Uppsala, Sweden). As a comparison, healthy volunteers ($n = 27$) underwent SPTs to the same allergens and to morphine.

Skin biopsy specimens and staining

Three-millimeter-diameter punch biopsy specimens of skin ($n = 5$) and autopsy skin specimens ($n = 3$) were obtained; all specimens were formalin fixed, routinely processed, and paraffin embedded, and sections were stained with hematoxylin and eosin for histopathologic interpretation. Five-micrometer-thick tissue sections were prepared and placed on poly-L-lysine–coated glass slides for immunohistochemistry. Immunohistochemistry was performed with anti-mast cell tryptase (clone T311, catalog no. NCL-L-TYROS, dilution 1:20, incubation time of 2 hours; Leica, Wetzlar, Germany) or anti-CD117 antibody (rabbit polyclonal, catalog no. A4502, dilution 1:200, incubation time of 2 hours; Dako, Glostrup, Denmark) and the Ultra View Detection System (Roche Ventana Medical Systems, Tucson, Ariz) on an automated system according to the manufacturer’s instructions (BenchMark ULTRA Automated Slide Stainer; Roche Ventana Medical Systems). Photomicrographic images were acquired with the Olympus DP71 digital camera attached to an Olympus BX41 microscope equipped with either a $\times 10$ objective lenses (UIS 2 UPlanSApo; Olympus, Center Valley, Pa) or a $\times 60$ objective lenses (UIS 2 UPlanFL N;

Olympus). Integrated densitometry was performed to quantify staining intensity (ImageJ software; National Institutes of Health, Bethesda, Md).

Bone marrow aspirate flow cytometric evaluation

Bone marrow aspirates were washed, red cells lysed, and subsequently stained with fluorochrome-conjugated antibodies against CD45, CD117, CD34, CD11b, CD38, and HLA-DR. Multiparameter flow cytometry was done with the FACSCanto II system (BD, San Jose, Calif). Flow cytometric data analysis was done with FCS Express 6 (De Novo Software, Glendale, Calif).

Primary mast cell culture and degranulation

CD34⁺ cells were positively selected from PBMCs using the ferromagnetic bead-based MACS system (Miltenyi Biotec, Bergisch Gladbach, Germany), and cell numbers were quantified. Mast cells were differentiated from these precursors under established conditions, as previously described.^{E22} IgE-dependent mast cell degranulation was accomplished by sensitizing cells with biotinylated human myeloma IgE (Calbiochem, San Diego, Calif) overnight (16 hours) in the absence of SCF (PeproTech, Rocky Hill, NJ) and subsequent cross-linking with streptavidin in the presence or absence of SCF (10 ng/mL), as described. Degranulation was quantified by measuring the percentage of β -hexosaminidase release or intracellular calcium flux by using Fura-2 AM ester (Molecular Probes, Eugene, Ore) at a fluorescent intensity ratio at 340 and 380 nm after background subtraction, as described.^{E23}

Primary mast cell imaging

Cytospin preparations of 7-week-old cultured human mast cells were fixed and stained with toluidine blue for microscopic examination, as described.^{E24}

LAD2 cell culture and degranulation

LAD2 cells were cultured in the presence of the GATA-2 inhibitor (GATA2i) K-7174^{E13} or in the presence of dimethyl sulfoxide (control) at indicated concentrations and durations. IgE-dependent degranulation was accomplished in the same manner as for primary mast cell cultures. IgE-independent degranulation was determined by treating mast cells with phorbol 12-myristate 13-acetate and ionomycin for 30 minutes. Degranulation was determined by means of flow cytometry with CD63 and CD107a (lysosomal-associated membrane protein 1).

Flow cytometry

PBMCs were isolated from whole blood using Ficoll-Hypaque gradient centrifugation. PBMCs were washed with PBS and resuspended in RPMI 1640 (Gibco, Carlsbad, Calif) supplemented with 10% heat-inactivated FCS (Gemini Bio-Products, West Sacramento, Calif), 100 U/mL penicillin G, 100 μ g/mL streptomycin, and 29.2 μ g/mL L-glutamine (Gibco). PBMCs and cultured LAD2 cells were stained with LIVE/DEAD Blue (Thermo Scientific, Waltham, Mass), and then protein surface expression on mast cells was determined by using anti-CD63 phycoerythrin (PE), anti-CD107a Pacific Blue (BD), anti-Fc ϵ RI BV605, anti-CD107a, and anti-CD117 PE-Dazzle 594 (BioLegend, San Diego, Calif), whereas PBMCs were stained by using anti-CD117 PE-Dazzle 594, anti-Fc ϵ RI BV605, anti-BDCA-2 fluorescein isothiocyanate (FITC; BD, San Jose, Calif), and anti-CD123 BV510 (BioLegend).

Droplet digital PCR

Total RNAs were extracted with TRIzol reagent (Thermo Scientific) and the RNEasy Mini Kit (Qiagen, Hilden, Germany) from mast cells under stated conditions and reverse transcribed by using the SuperScript III First-Strand Synthesis System (Thermo Scientific). Droplet digital PCR was performed on a QX200 system (Bio-Rad Laboratories, Hercules, Calif), according to the manufacturer’s instructions, to quantitate *FCER1A*, *KIT*, *GATA2* (Bio-Rad Laboratories), *CXCL2*, and *IL6ST* (Thermo Scientific) gene expression using *HPRT1* and *TBP* (Bio-Rad Laboratories) expression as references and using TaqMan primer/probe sets.

Bioinformatics

Public gene counts (SRP048759) were downloaded by using the R package *recount*.^{E25} Genes with read counts of less than 5 in the majority of samples were filtered out. Differential gene expression analyses were performed with DESeq2.^{E26}

Statistical analyses

The Fisher exact test was used to compare population and clinical prevalence data. Unpaired and paired 2-tailed *t* tests and 2-tailed Mann-Whitney tests were applied to test significance, where appropriate, as stated in the text. All tests were performed with Prism software (GraphPad Software, La Jolla, Calif).

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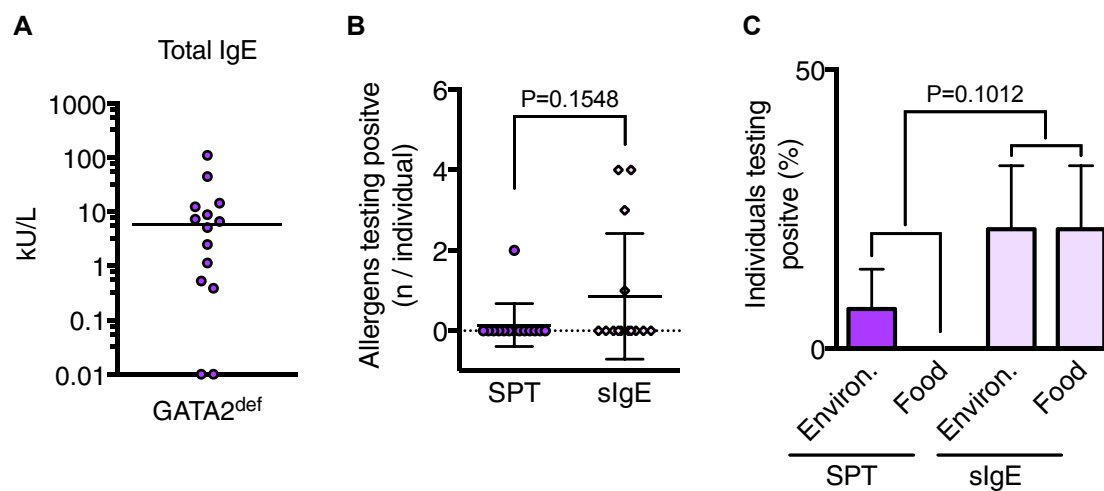


FIG E1. Serum IgE levels and specificity in GATA-2 deficiency. **A**, Total IgE levels from GATA-2-deficient subjects (n = 14). **B** and **C**, Positive antigen-specific serum IgE (*sIgE*) numbers (Fig E1, **B**) and percentages of subjects (Fig E1, **C**) to 11 environmental and 8 food allergens tested. The Fisher exact test was used.

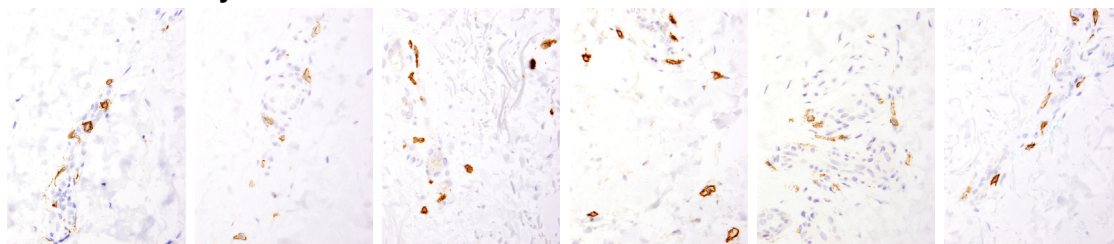
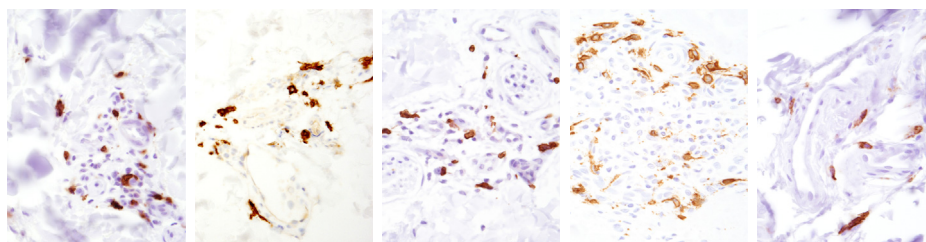
A GATA2 deficiency**B Control**

FIG E2. Reduced KIT staining in skin mast cells from GATA-2-deficient patients. Immunohistochemical staining of KIT in skin punch biopsy specimens from 6 subjects with GATA-2 deficiency (**A**) and 5 GATA-2 wild-type (control) subjects (**B**).

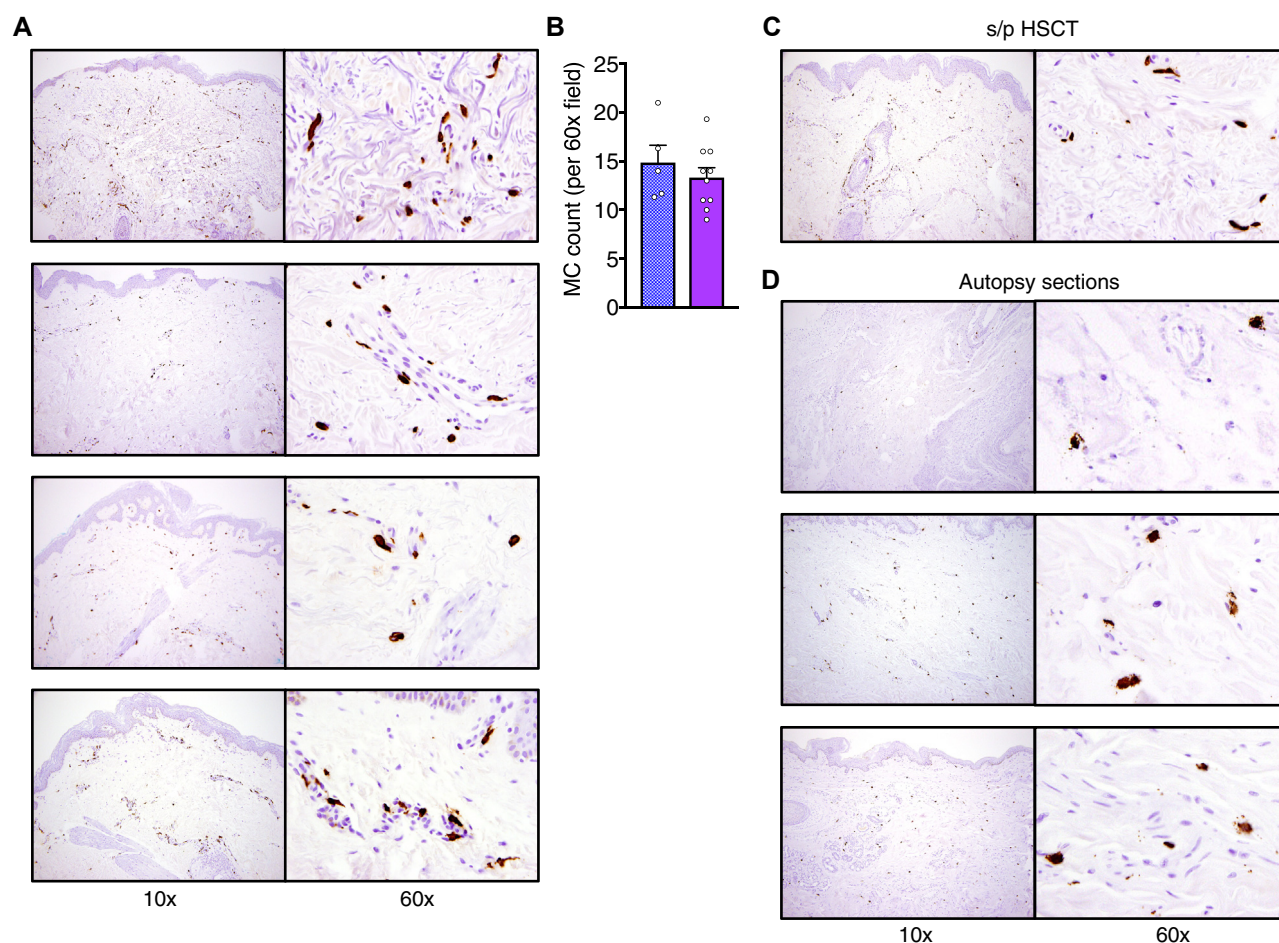


FIG E3. Skin mast cells in subjects with GATA-2 deficiency. Representative low-power ($\times 10$) and high-power ($\times 60$) photomicrographs of tryptase immunohistochemistry staining cutaneous mast cells (MCs) from GATA-2-deficient subjects before (**A**; quantification in **B**) and after (**C**) hematopoietic stem cell transplantation (HSCT) and in autopsy specimens from subjects not undergoing transplantation (**D**).

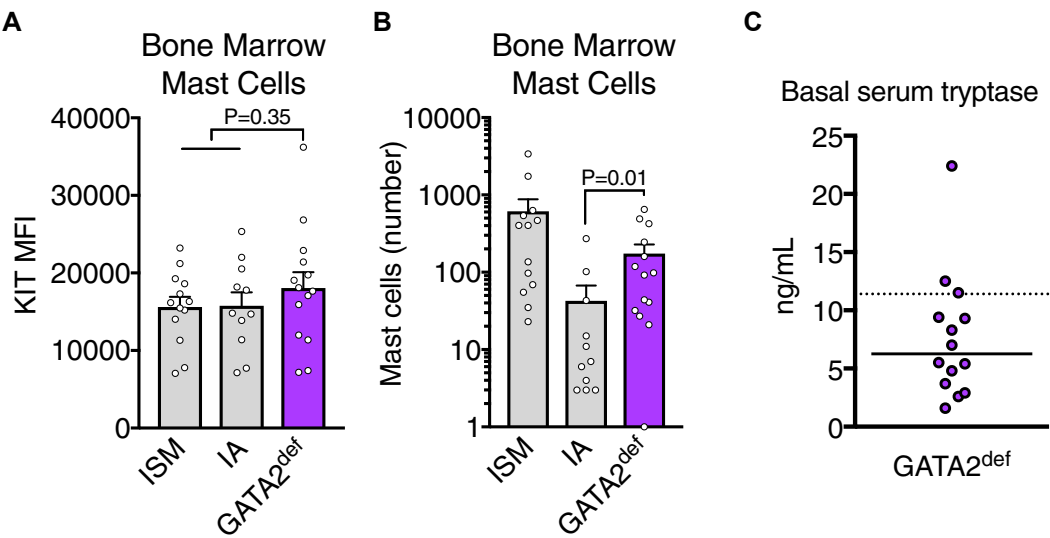


FIG E4. Bone marrow mast cells and basal serum tryptase in subjects with GATA-2 deficiency. KIT expression (**A**), mast cell number (**B**), and basal serum tryptase (**C**). The Mann-Whitney test was used. *IA*, Idiopathic anaphylaxis; *ISM*, indolent systemic mastocytosis; *MFI*, mean fluorescent intensity.

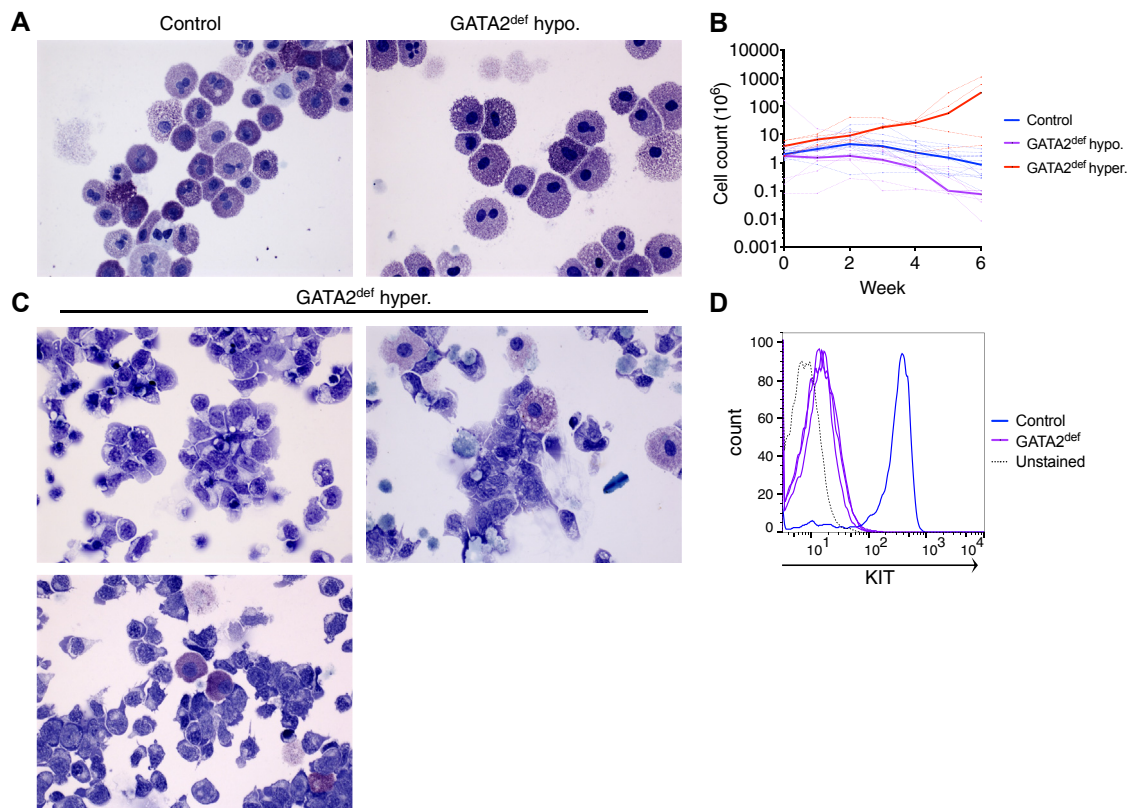


FIG E5. Primary mast cell cultures reveal disruption of mast cell phenotypes in subjects with GATA-2 deficiency. **A**, Representative photomicrographs from successful primary mast cell cultures from a GATA-2-deficient subject and a paired control subject. **B**, Combined growth curves (averaged based upon growth characteristics in dark lines) from 12 independent cultures (*dashed lines*) of CD34⁺ cells. Successful mast cell cultures hypoproliferated (*hypo.*) in purple relative to control cultures in blue; 3 cultures hyperproliferated (*hyper.*) and displayed dysplastic features (**C**). **D**, KIT surface staining of GATA-2-deficient cells from Fig E5, **C**, and a representative paired control subject.

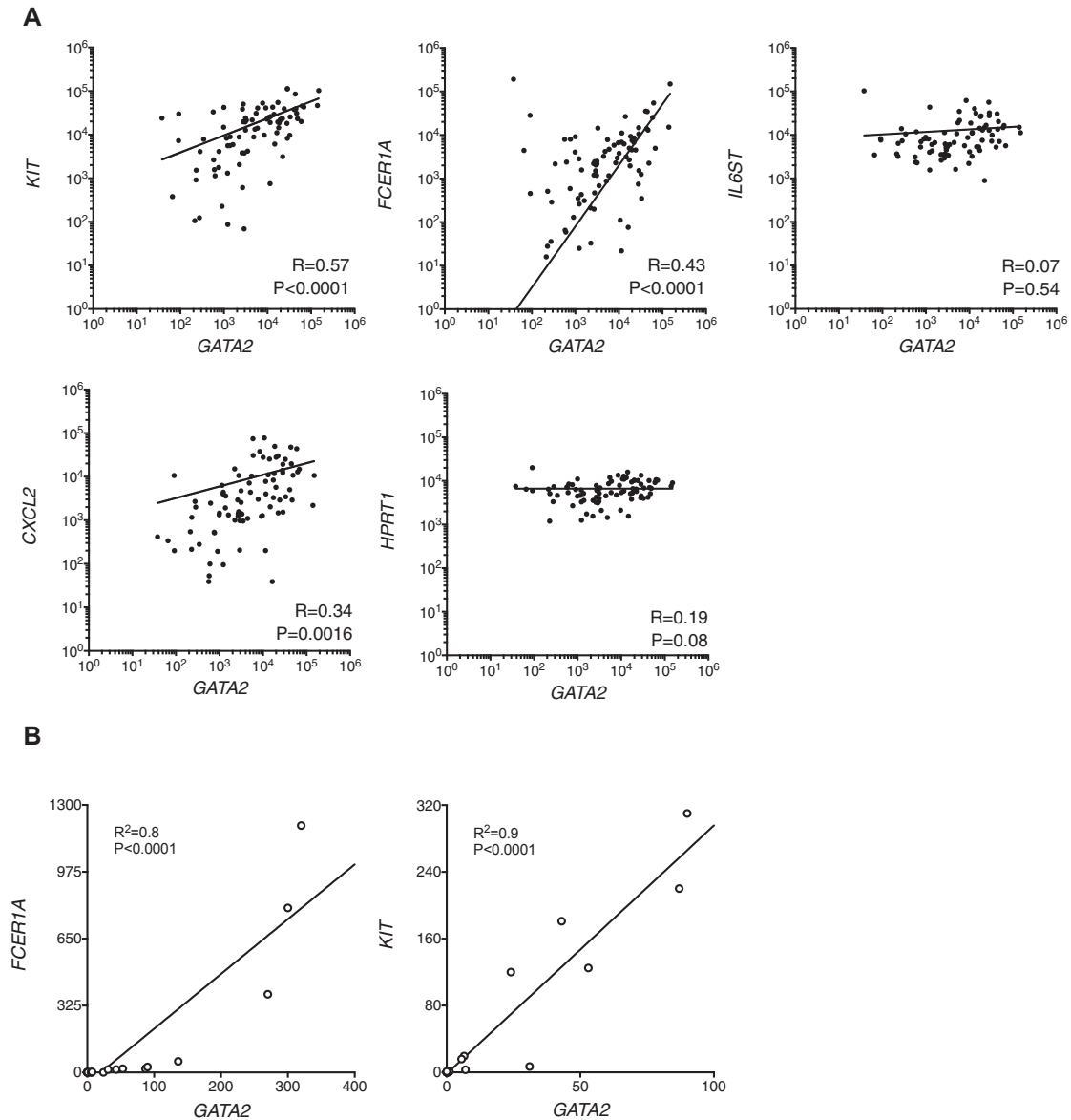


FIG E6. Graded *GATA2* expression positively correlates with *KIT* and *FCER1A* expression. **A**, Analysis of RNA sequencing data demonstrating correlation of *GATA2* transcript levels and targets examined in this study (top) and correlation between *GATA2* and a reported target (*CXCL2*) and unrelated gene commonly used as a low-abundance reference (*HPRT1*). **B**, Droplet digital PCR-quantified gene expression in cDNAs from primary cultured mast cells, isolated basophils, and transformed mast cell lines.

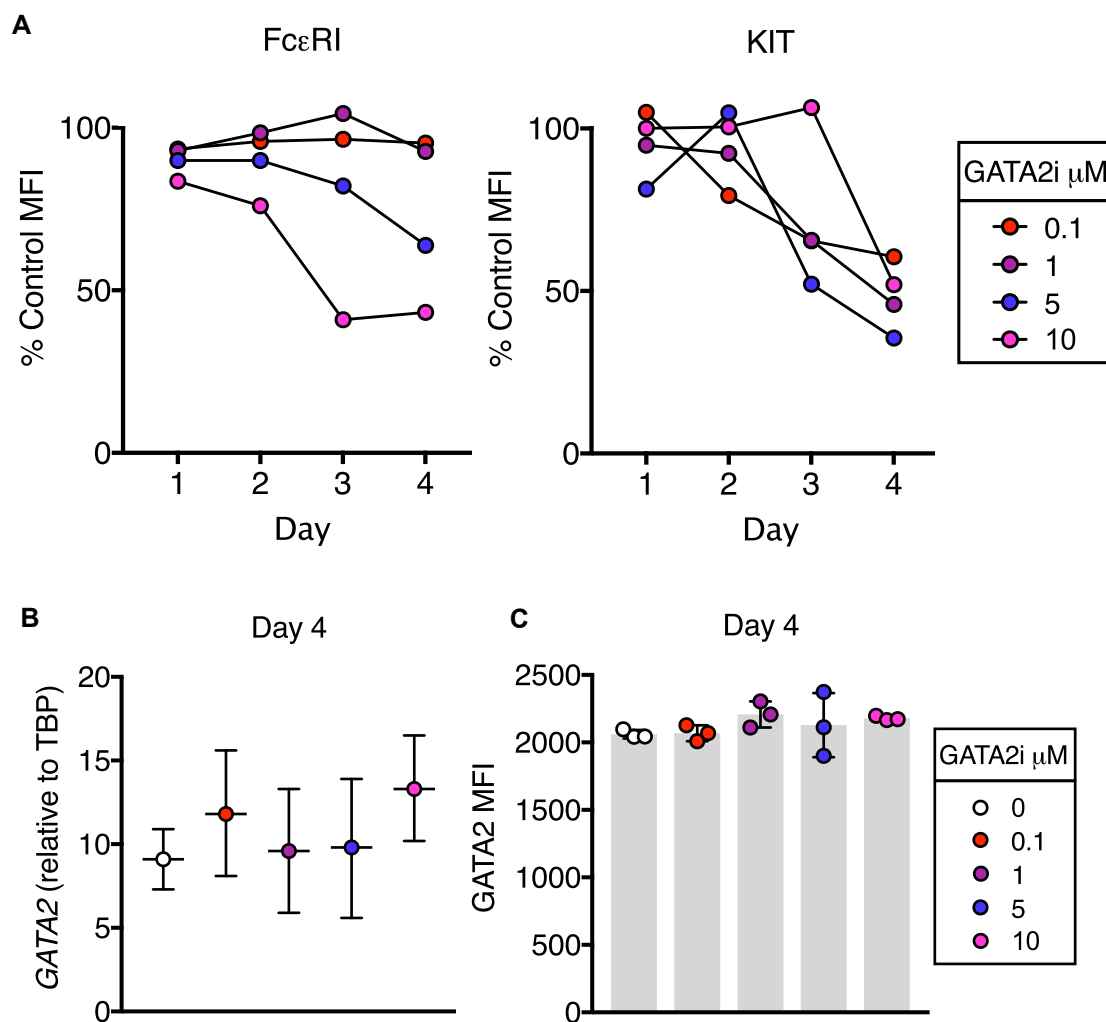


FIG E7. Temporal and dose responses of FcεRI and KIT to GATA-2 inhibition. Relative surface mean fluorescent intensity (MFI) of FcεRI and KIT after LAD2 cell culture with dimethyl sulfoxide or the GATA-2 inhibitor (GATA2i) K-7174 at indicated concentrations and time points (**A**) and relative expression levels of GATA-2 transcript (**B**) and protein (**C**) on day 4 at indicated inhibitor concentrations are shown.

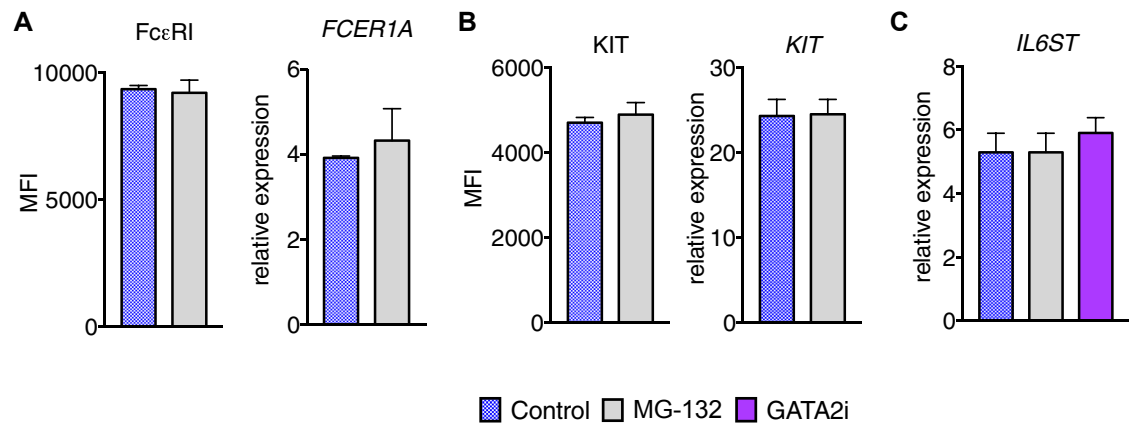


FIG E8. Gene expression in LAD2 cells. **A** and **B**, Surface protein expression (*left*) and gene expression (*right*) of FcεRI encoded by *FCER1A* (Fig E8, **A**) and KIT encoded by *KIT* (Fig E8, **B**) in LAD2 cells after treatment with the proteasome inhibitor MG-132 (4 μmol/L) for 4 days. **C**, *IL6ST* gene expression in LAD2 cells after culture under the same conditions or in the presence of GATA2i. A paired 2-tailed *t* test was used.

TABLE E1. Allergy medication query list

Accolate (zafirlukast)
Alocril (nedocromil)
Azelastine
Cetirizine
Chlorpheniramine
Clarinet (desloratidine)
Cromolyn
Cyproheptadine
Desloratidine
Diphenhydramine
Doxylamine
Epinephrine
Fexofenadine
Hydroxyzine
Levocetirizine
Loratadine
Montelukast
Nedocromil
Periactin (cyproheptadine)
Tilade (nedocromil)
Xyzal (levocetirizine)
Zafirlukast
Zileuton
Zyflo (zileuton)

Exact search terms queried based upon formulary availability of both brand name and generic medications at the NIH clinical center.

TABLE E2. Patients' phenotypic data

Subject no.	Sex	GATA-2 mutation	Clinical characteristics	Tryptase (ng/mL)	IgE (kU/L)	Mast cell culture	Allergy history		SPT Allergen	Antigen-specific IgE (kU/L [>0.1])
							Personal	Unaffected Family member		
1	M	c.1017+572C>T	78 y/o white man, otherwise healthy, found to have early CMML	7	7.35	Hyper; granulated and mature	Seasonal allergic rhinitis	None	Negative	ND
2	F	c.1009C>T; p.R337X	44 y/o white woman with recurrent sepsis, lower-extremity edema, panniculitis, refractory warts, autoimmune thyroiditis, chronic osteomyelitis, thrombosis, hearing loss, and oral ulcers	3.7	8.85	Hyper; agranular, cytokine independent	None	M-Hymenoptera	Negative	ND
3	M	c.1163C>T; p.M388T	76 y/o white man with recurrent squamous cell cancer, coronary artery disease, Grave disease, gout, hyperlipidemia, reflux, neuropathy, renal cell cancer, hypertension, and COPD	5.5	1.14	Hyper; agranular, cytokine independent	None	GC-FA	Negative	ND
4	F	c.1187G>A; p.R396Q	25 y/o white woman with trisomy 8, disseminated MAI, MDS	5.4	2.50	Hyper; agranular, cytokine independent	None	F-AR	Negative	ND
5	F	c.302delG; p.G101AfsX16	22 y/o black woman with trisomy 8, AML s/p chemotherapy age 14 y, hypogammaglobulinemia, disseminated CMV, extensive HSV2, lymphedema, chronic sinusitis, CMML (died day of transplantation)	9.3	6.64	Hypo; granulated and mature	Seasonal allergic rhinitis	M-AA, AR, F-AR	Negative	Timothy, 1.11; birch, 0.16; oak, 0.28
6	F	c.988C>T; p.R330X	25 y/o white woman with trisomy 8, refractory warts, and chronic EBV infection	9.4	0.01	Hypo; none survived	None	M-AR; F-AR	Negative	ND
7	F	c.988C>T; p.R330X	25 y/o white woman with trisomy 8, aphthous ulcers, refractory warts requiring vulvectomy, lower-extremity edema, and panniculitis	12.5	0.01	Hypo; none survived	None	M-AR; F-AR	Negative	ND
8	F	c.1113C>G; p.N371K	39 y/o white woman with disseminated MAC, DVT, embolic CVA, extensive HPV, panniculitis, hearing loss, splenectomy, h/o miscarriages, severe <i>Clostridium difficile</i> , esophageal candidiasis, MDS s/p BMT	11.5	12.40	Hypo; granulated and mature	None	None	Negative	Milk, 0.13
9	M	c.1061C>T; p.T354M	34 y/o Hispanic man with trisomy 8, disseminated MAI, PAP, anal condyloma, MDS s/p BMT	1.6	111.00	Hypo; granulated and mature	None	None	Cockroach; <i>Dermatophagoides farinae</i>	<i>Dermatophagoides pteronyssinus</i> , 0.13; shrimp, 0.45; cockroach, 0.47; <i>Aspergillus</i> species, 0.17

(Continued)

TABLE E2. (Continued)

Subject no.	Sex	GATA-2 mutation	Clinical characteristics	Tryptase (ng/mL)	IgE (kU/L)	Mast cell culture	Allergy history		SPT Allergen	Antigen-specific IgE (kU/L [>0.1])
							Personal	Unaffected Family member		
10	F	c.1018-1G>A; p.S340_D381del	47 y/o Caucasian female with trisomy 1, disseminated MAC, zoster, hearing loss, panniculitis, MDS s/p BMT x2 (had recurrent clone)	2.6	44.90	hypo; granulated and mature	None	None	Neg	Der p 0.37; Der f 0.22; Shrimp 0.20; Cockroach 0.35
11	M	c.1198C>T; p.R398W	22 y/o white woman with disseminated <i>Mycobacterium fortuitum</i> , MDS, lacunar infarct, and warts	8.3	0.53	Hypo; granulated and mature	None	None	Negative	ND
12	M	c.1186C>T; p.R396W	24 y/o Asian man with MAI, warts, lymphadenopathy, EBV-associated smooth muscle neoplasm, increased tryptase level	22.4	5.15	Hypo; granulated and mature	None	M-FA	Negative	ND
13	M	c.1017+572C>T	30 y/o white man, family member of subject 1, has lymphedema but no warts and normal numbers of monocytes, B cells with low NK cell counts	4.8	14.40	Hypo; granulated and mature	None	F-AR, OAS	Negative	ND
14	F	c.1017+572C>T	28 y/o white woman; asymptomatic family member of subject 1; born premature at 26 wk; diagnosis of cerebral palsy; history of warts and thrush on 2 occasions; normal numbers of monocytes, B cells, and low NK cell counts	2.9	0.39	Hypo; granulated and mature	None	F-AR, OAS	Negative	ND

AA, Allergic asthma; AML, acute myeloid leukemia; AR, allergic rhinitis; BMT, bone marrow transplantation; CMML, chronic myelomonocytic leukemia; CMV, cytomegalovirus; COPD, chronic obstructive pulmonary disease; CVA, cerebrovascular accident; DVT, deep vein thrombosis; F, female; FA, food allergy; GC, grandchild; HPV, human papillomavirus; h/o, history of; HSV2, herpes simplex virus type 2; Hyper, hyperproliferated; Hypo, hypoproliferated; M, mother; MAC, mycobacterium avium complex; MAI, mycobacterium avium-intracellulare infection; MDS, myelodysplastic syndrome; ND, not determined; OAS, oral allergy syndrome; PAP, pulmonary alveolar proteinosis; s/p, status-post.

TABLE E3. Selected allergen SPT panel

<i>Alternaria tenuis</i>
<i>Aspergillus fumigatus</i>
Birch mix
Cashew nut
Cat hair
Cockroach mix
<i>Dermatophagoides farinae</i>
<i>Dermatophagoides pteronyssinus</i>
Dog wander
Egg white
Whole milk
Mouse
Oak mix
Peanut
Ragweed mix
Shrimp
Soybean
Timothy
Black walnut
Wheat