IgG Fc glycosylation as an axis of humoral immunity in childhood

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We thank Ms Corinne Kwek for her assistance with the subjects, the subjects for their participation, and Linqiu Cao and Mireille Gadella (FrieslandCampina, Amersfoort, The Netherlands) for providing the enriched DP4-GOS sample.

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This work was supported by the National Medical Research Council (grants NMRC/CIRG/1368/2013 and NMRC/CIRG/1487/2018).

Disclosure of potential conflict of interest: D. J. Delsing is employed by FrieslandCampina, Amersfoort, The Netherlands. The rest of the authors declare that they have no relevant conflicts of interest.

REFERENCES

1. Vandenplas Y, Zakharova I, Dmitrieva Y. Oligosaccharides in infant formula: more evidence to validate the role of prebiotics. Br J Nutr 2015;113:1339-44.
2. Vo TH, Le NH, Patel MS, Phan LT, Tran Minh NN. Acute allergic reactions in Vietnamese children after drinking a new milk product. Foodborne Pathog Dis 2012;9:156-9.
3. Chiang WC, Huang CH, Llanora GV, Gerez I, Goh SH, Shek LP, et al. Anaphylaxis to cow's milk formula containing short-chain galacto-oligosaccharide. J Allergy Clin Immunol 2012;130:1361-7.
4. Soh JY, Chiang WC, Huang CH, Woo CK, Ibrahim I, Heng K, et al. An unusual cause of food-induced anaphylaxis in mothers. World Allergy Organ J 2017;10:3.
5. Coulier L, Timmermans J, Bas R, Van Den Dool R, Haaksman I, Klarenbeek B, et al. In-depth characterization of prebiotic galacto-oligosaccharides by a combination of analytical techniques. J Agric Food Chem 2009;57:8488-95.
6. Al-Ghouleh A, Johal R, Sharquie IK, Emara M, Harrington H, Shakib F, et al. The glycosylation pattern of common allergens: the recognition and uptake of Der p 1 by epithelial and dendritic cells is carbohydrate dependent. PLoS One 2012;7:e33929.
7. Halim A, Carlsson MC, Madsen CB, Brand S, Moller SR, Olsen CE, et al. Glycoproteomic analysis of seven major allergenic proteins reveals novel post-translational modifications. Mol Cell Proteomics 2015;14:191-204.
8. Commins SP, James HR, Kelly LA, Pochan SL, Workman LJ, Perzanowski MS, et al. The relevance of tick bites to the production of IgE antibodies to the mammalian oligosaccharide galactose-alpha-1,3-galactose. J Allergy Clin Immunol 2011;127:1286-93.e6.
9. Jyo T, Kuwabara M, Kodommari Y, Tanemori N, Asaoku Y, Katsutani T, et al. Cases of immediate type allergy in oyster shuckers due to galactooligosaccharide. Med J Hiroshima Prefectural Hospital 1993;25:19-27.

Available online October 15, 2019.
https://doi.org/10.1016/j.jaci.2019.10.003

IgG Fc glycosylation as an axis of humoral immunity in childhood

To the Editor:

In children, antibody levels and repertoire diversity mature with age. A further determinant of humoral immunocompetence, still poorly characterized in the pediatric population, is IgG Fc glycosylation. Paired biantennary glycans at asparagine 297 in each heavy chain modulate the ability of IgG to bind complement, Fcγ receptors, and other ligands. The availability of more than 30 glycoforms provides the opportunity to "fine-tune" IgG effector capacity.[1] Variation in IgG Fc glycan use with age is recognized, but small sample sizes and contrasting reports leave the pediatric end of this spectrum poorly defined.[2-6] We sought to test the hypothesis that IgG Fc glycans vary predictably with age in childhood and to explore the possibility that altered IgG Fc glycosylation might contribute to otherwise unexplained respiratory tract infections in children.

We obtained blood samples from 267 children aged 9 months to 18 years: 145 healthy children from Boston Children's Hospital, 82 healthy children from Erasmus Medical Center (Rotterdam, The Netherlands), and 40 children evaluated at the Immunology Clinic at Boston Children's Hospital for unexplained recurrent respiratory tract infections (RRI; see the Methods section in this article's Online Repository at www.jacionline.org for recruitment procedures). Unexplained RRIs were defined as recurrent infections of the middle ear, sinuses, and/or lung sufficient to trigger immunologic consultation during which the attending immunologist elected to perform quantitative assessment of humoral immunity but for which no cause was ultimately determined. Patients were excluded if RRIs were attributed to an environmental cause; a predisposing condition, such as cystic fibrosis; or a diagnosable immunodeficiency, such as hypogammaglobulinemia, IgG subclass deficiency, specific antibody deficiency, or a defect in complement or cellular immunity (see Table E1 in this article's Online Repository at www.jacionline.org). Patients with IgA levels of less than the lower limit of normal in the absence of other immune abnormalities were described as "low IgA" and evaluated as a distinct subgroup (see Table E2 in this article's Online Repository at www.jacionline.org). Subjects receiving immunoglobulin supplementation at any point before sampling were excluded.

IgG Fc glycans were quantitated using liquid chromatography and electrospray ionization mass spectrometry (LC-ESI-MS). Glycopeptide fragments from IgG_2 and IgG_3 are indistinguishable by this method and are reported together. Methods and statistical strategies are provided in the Methods section in this article's Online Repository.

Individual glycoforms, as well as summary measurements of galactosylated, sialylated, bisected, and fucosylated glycans, were plotted as continuous variables and by age group: 9 months to 2 years (n = 59), 2 to 5 years (n = 76), and 5 to 18 years (n = 92). Galactosylation exhibited age-dependent variability consistent across IgG subclasses (Fig 1, *A*, and see Figs E1-E4 in this article's Online Repository at www.jacionline.org). Digalactosylated (G2) forms were overrepresented in younger children, largely at the expense of monogalactosylated (G1) glycans. The agalactosylated (G0) fraction was highest in children aged 2 to 5 years. Younger children exhibited more sialylated and core-fucosylated glycans (Fig 1, *A*), which are typically considered anti-inflammatory, as well as alteration in glycans of unclear functional effect, including those featuring a bisecting *N*-acetylglucosamine or variant hybrid structures (IgG_1 is shown in Fig 1, *A*; Figs E1 and E4 show other isotypes). Thus, compared with older children, younger children exhibited a distinct pattern of IgG Fc glycosylation.

To test whether IgG Fc glycans varied in a regular manner, we assessed the ability of combinations of glycoforms to differentiate children by age. An elastic net multinomial classifier was trained in the Boston healthy cohort, yielding a classification accuracy of approximately 80% across age categories (Fig 1, *B*). Validation in the Dutch cohort confirmed

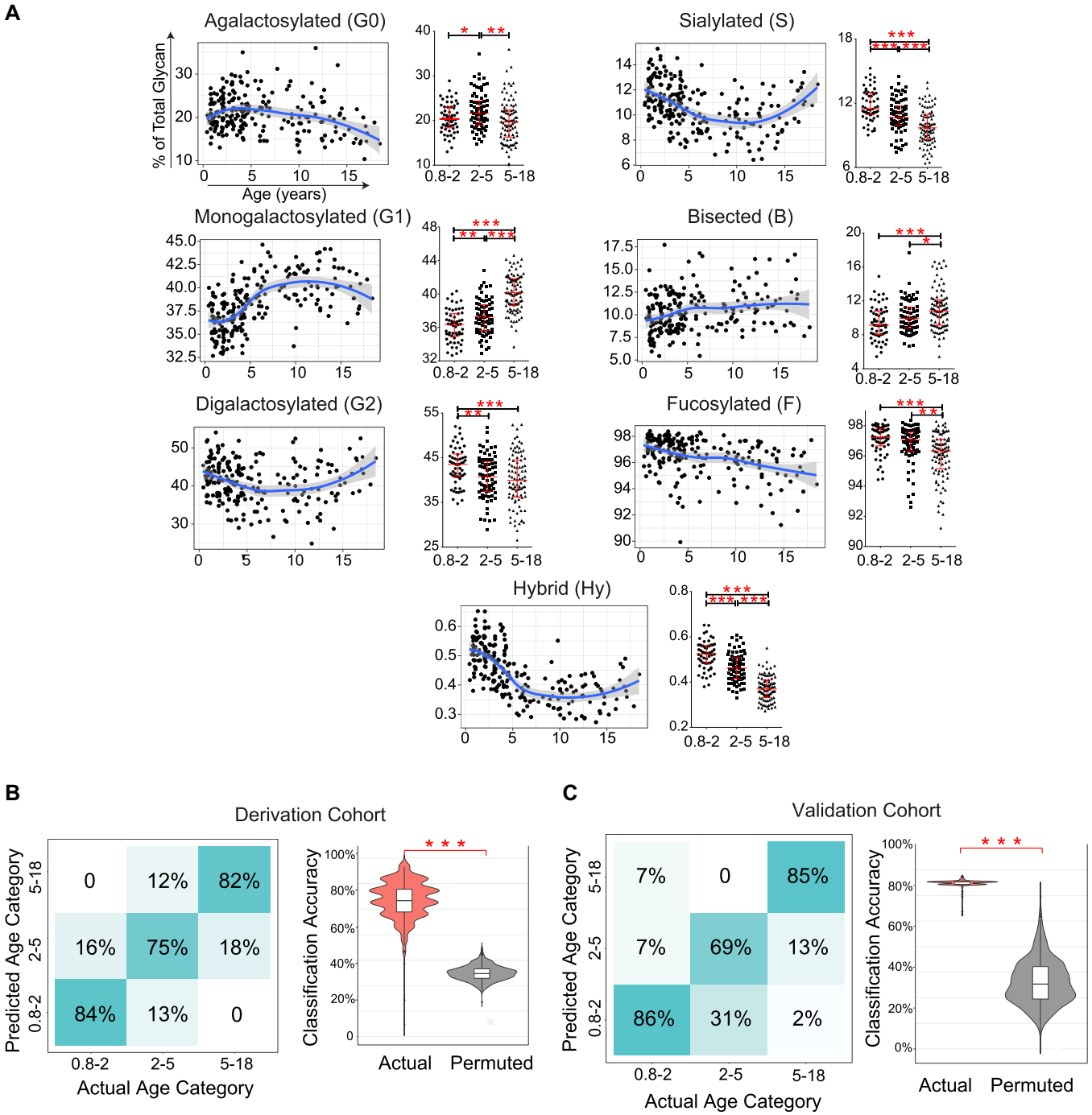

FIG 1. IgG_1 Fc glycans in healthy children. **A,** Levels of galactosylated, sialylated, bisected, fucosylated, and hybrid IgG_1 glycans in 145 healthy children, as defined by using LC-ESI-MS plotted by age. See the Methods section in this article's Online Repository for other IgG isotypes. **B,** Accuracy of age classification of the Boston cohort using Fc glycosylation profiles in the derivation Boston cohort (*left*, n = 145). **C,** Validation of age classification by Fc glycosylation profile in the Dutch cohort (*right*, n = 82) using actual versus permuted data. *P* values were adjusted in Fig 1, *A*, for multiple comparisons: $*P < .05$, $**P < .01$, and $***P < .001$.

80% accuracy (Fig 1, *C*), demonstrating a robust and generalizable relationship between IgG Fc glycosylation and age in children. Female subjects older than the average age of menarche (≥12 years) showed enhanced Fc galactosylation compared with their similarly aged male counterparts, which is consistent with the known effect of estrogen on IgG glycans (see Fig E5 in this article's Online Repository at www.jacionline.org).[7] These findings link age and sex to predictable changes in IgG Fc glycosylation in childhood.

To explore the role of IgG Fc glycosylation in susceptibility to infection, we performed LC-ESI-MS in 40 patients with unexplained RRIs together with 3 age-matched healthy control

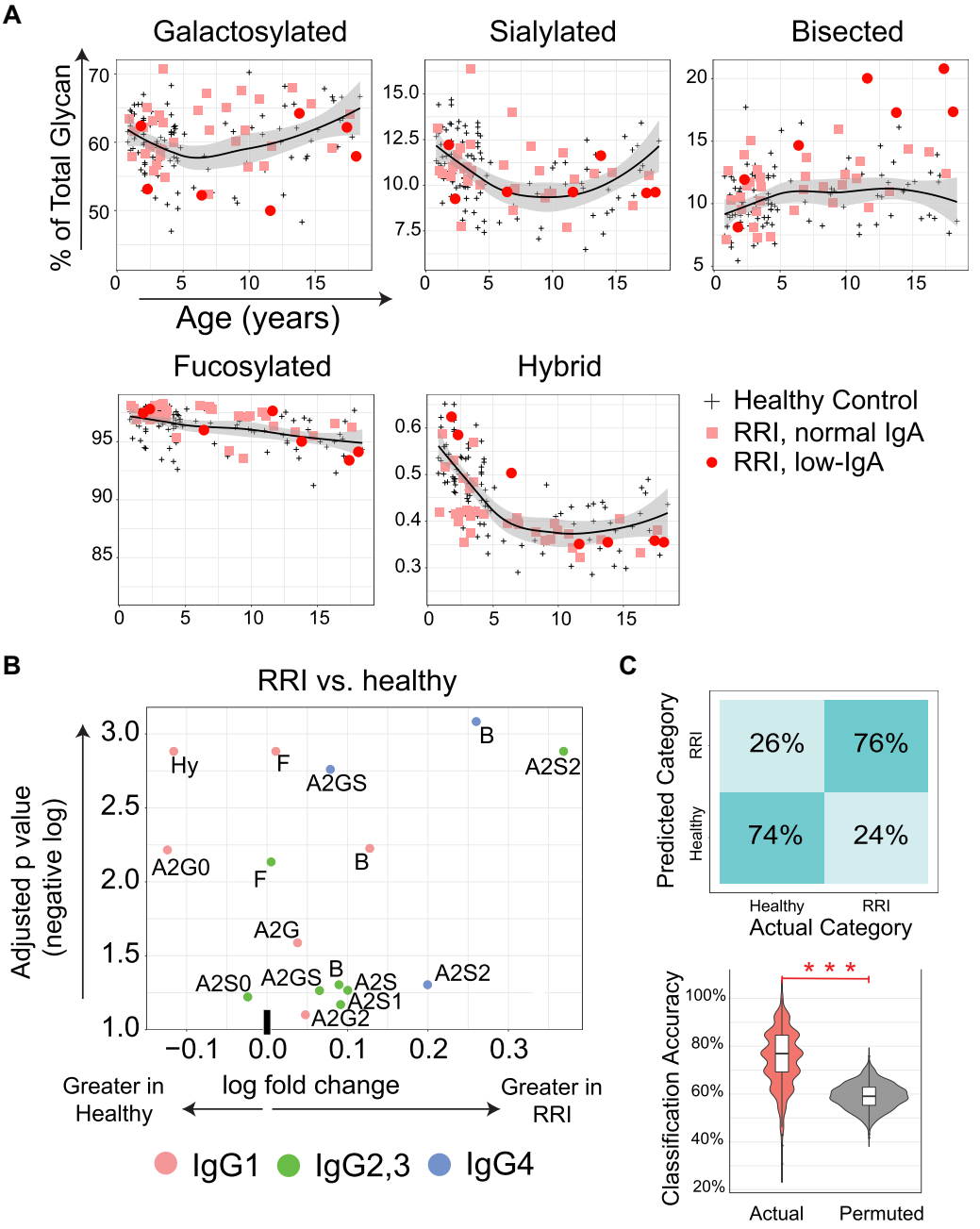

FIG 2. IgG Fc glycans in healthy children and patients with recurrent respiratory infection (RRI). **A,** Scatterplots of galactosylated, sialylated, bisected, fucosylated, and hybrid glycans by age in IgG_1 from patients with RRIs with normal IgA levels (n = 33), patients with RRIs with low IgA (n = 7), and age-matched healthy children (n = 120; black locally weighted scatterplot smoothing line, 95% CI is indicated in gray). **B,** Volcano plot depicting differences between healthy subjects and patients with RRIs. *A2*, Biantennary; *B*, bisected; *G*, galactosylated (0, 1, or 2); *Hy*, hybrid; *S*, sialylated (0, 1, or 2). **C,** Accuracy of classification model distinguishing healthy subjects from patients with RRIs trained by using actual data (pink) compared with permuted data (gray). ***$P < .001$.

subjects per case to eliminate batch effects (n = 120). Cases showed increased levels of bisected glycans across all subclasses, as well as enhanced sialylation of $IgG_{2/3}$ and IgG_4 (Fig 2, *A* and *B*, and see Fig E2). Binomial classifiers using glycan data categorized 74% of patients with RRIs and healthy subjects correctly ($P < .001$ when compared with permuted data; Fig 2, *C*). Although the number of patients with low IgA was limited, an especially marked abundance of bisected forms was noted in this subpopulation (see Fig E6 in this article's Online Repository at www.jacionline.org). Thus otherwise unexplained RRIs in children are associated with IgG Fc glycan aberrancy.

Fc glycans modulate IgG effector capacity, and it is likely that variation in healthy children and in those with RRIs will

have implications for immune function. Contrasting reports of the effect of individual glycoforms on IgG function and simultaneous variation across a wide spectrum of glycoforms complicate prediction of the net functional effect of the changes observed here.[1] In healthy children the abundance of digalactosylated and sialylated forms, as well as enhanced core fucosylation, could attenuate the effector potency of the IgG pool, especially before the age of 2 years. Whether the glycan changes noted here in children with unexplained RRIs represent a predisposing factor or an effect of frequent infections cannot be determined from this data set. However, chronic immune stimulation is typically associated with lower levels of sialylation, galactosylation, or both, a pattern distinct from and even opposite that observed here.[3,8,9] If IgG Fc glycan aberrancy predisposes to RRIs in some children, then an intriguing implication is that immunoglobulin supplementation addresses not only abundance and repertoire but also the deficit in IgG bearing normal Fc glycans and therefore with normal effector function.

Assessment of IgG Fc glycans is not part of the routine immunologic evaluation. Our data suggest that IgG Fc glycosylation represents an axis of humoral immune regulation in childhood and raise the intriguing possibility that IgG glycan aberrancy could contribute to otherwise unexplained respiratory tract infections in some patients. These findings highlight the need to consider the effect of IgG Fc glycans on immunocompetence in childhood and beyond.

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L.D.N. was supported by the Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health. R.S. was supported by funding from the Science Foundation Ireland Starting Investigator Research Grant (SFI SIRG) under grant number 13/SIRG/2164. H.S. and R.O'F. were supported by the funding from EU FP7 Program HighGlycan, grant number 278535. M.E.A. was supported by NIH grants P20GM104416, R01AI131975, and P01AI120756 and an Innovative Research Award from the Rheumatology Research Foundation. P.A.N. was supported by NIH grants R21AI099435, R01AR065538, R01AR073201, R01AR075906, and P30AR070253; an Innovative Research Award from the Rheumatology Research Foundation; the Cogan Family Foundation; the Fundación Bechara; and the Arbuckle Family Fund for Arthritis Research.

Disclosure of potential conflict of interest: L. D. Notarangelo was supported by the Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH). M. E. Ackerman was supported by NIH grants P20GM104416, R01AI131975, and P01AI120756 and an Innovative Research Award from the Rheumatology Research Foundation. P. A. Nigrovic was supported by NIH grants R21AI099435, R01AR065538, R01AR073201, R01AR075906, and P30AR070253; an Innovative Research Award from the Rheumatology Research Foundation; the Arbuckle Family Fund for Arthritis Research; and the Fundación Bechara. The rest of the authors declare that they have no relevant conflicts of interest.

REFERENCES

1. Dekkers G, Rispens T, Vidarsson G. Novel concepts of altered immunoglobulin G galactosylation in autoimmune diseases. Front Immunol 2018;9:553.
2. Parekh R, Roitt I, Isenberg D, Dwek R, Rademacher T. Age-related galactosylation of the N-linked oligosaccharides of human serum IgG. J Exp Med 1988;167:1731-6.
3. Ercan A, Barnes MG, Hazen M, Tory H, Henderson L, Dedeoglu F, et al. Multiple juvenile idiopathic arthritis subtypes demonstrate pro-inflammatory IgG glycosylation. Arthritis Rheum 2012;64:3025-33.
4. Pucic M, Muzinic A, Novokmet M, Skledar M, Pivac N, Lauc G, et al. Changes in plasma and IgG N-glycome during childhood and adolescence. Glycobiology 2012; 22:975-82.
5. Kristic J, Vuckovic F, Menni C, Klaric L, Keser T, Beceheli I, et al. Glycans are a novel biomarker of chronological and biological ages. J Gerontol A Biol Sci Med Sci 2014;69:779-89.
6. de Haan N, Reiding KR, Driessen G, van der Burg M, Wuhrer M. Changes in healthy human IgG Fc-glycosylation after birth and during early childhood. J Proteome Res 2016;15:1853-61.
7. Ercan A, Kohrt WM, Cui J, Deane KD, Pezer M, Yu EW, et al. Estrogens regulate glycosylation of IgG in women and men. JCI Insight 2017;2:e89703.
8. Kaneko Y, Nimmerjahn F, Ravetch JV. Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. Science 2006;313:670-3.
9. Ackerman ME, Crispin M, Yu X, Baruah K, Boesch AW, Harvey DJ, et al. Natural variation in Fc glycosylation of HIV-specific antibodies impacts antiviral activity. J Clin Invest 2013;123:2183-92.

Available online October 24, 2019.
https://doi.org/10.1016/j.jaci.2019.10.012

Persistent cow's milk allergy is associated with decreased childhood growth: A longitudinal study

To the Editor:

Food allergy is a prevalent problem, with well-described risks involving safety, comorbidities, and quality of life. An expanding body of research suggests that food allergy and elimination diets can also affect childhood growth and nutrition and that allergy to cow's milk (CM)[1-4] or multiple foods[5] impart particular risk. Nearly all studies to date have been cross-sectional, and the few longitudinal investigations of growth in children with food allergy have been limited to the first years of life.[6,7] Data are lacking on growth patterns for children with ongoing food allergy that persists after the early childhood years, raising questions about the long-term effect on body morphology and adult height. Here we present the first description of growth in a longitudinal cohort of children with persistent food allergy that extends from early childhood to adolescence.

A retrospective chart review of patients from the Johns Hopkins Pediatric Allergy Clinic was completed for children with a diagnosis of either persistent CM or peanut and/or tree nut (PN/TN) allergy (and no known history of CM allergy). *Persistent food allergy* was defined as physician-diagnosed IgE-mediated

METHODS

Study approval

Boston healthy control and immunodeficient samples were collected under Boston Children's Hospital Institutional Review Board–approved protocols NS10-11-0578 and P00007449 (discarded samples) and 04-04-051 (written informed consent from legal guardians). Dutch healthy control samples were collected with approval of the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam, The Netherlands (MEC-2005-137), with written informed consent from legal guardians.

Healthy children

Plasma from healthy children was obtained from (1) healthy patients recruited from Boston Children's Hospital (n = 119),[E1] (2) laboratory discards (n = 26),[E1] and (3) consented healthy children from Erasmus Medical Center (n = 82).[E2,E3]

Immunodeficient children

Discarded plasma was collected from children in whom quantitative IgG subtype testing had been ordered from the Immunology Clinic at Boston Children's Hospital. Medical record review was performed within 1 month to identify subjects meeting the criteria for an unexplained RRI (see main text) and to collect nonidentifying demographic, laboratory, and clinical data. All identifiers were then destroyed.

LC-ESI-MS

Total IgG was isolated from human plasma, as described previously.[E4] Dried antibodies were dissolved in 40 μL of 25 mmol/L ammonium bicarbonate buffer (pH 7.9) containing 0.025 mg/mL TPCK-treated trypsin and incubated overnight at 37°C. Tryptic IgG digests were separated by using reversed-phase liquid chromatography and analyzed by using mass spectrometry, as described previously.[E4]

Data processing, including analyte peak integration and data curation, was performed with LaCyTools v1.0.[E5] Data were converted to mzXML files, and runs were aligned based on the exact mass and average retention time over all runs of the 3 most abundant glycoforms of each IgG subclass. Using the described separation methods, glycopeptides were separated in 3 glycopeptide clusters, one for each of the IgG subclasses IgG_1 and IgG_4 and one for the combination of IgG_2 and IgG_3, because these result in indistinguishable glycopeptides. After alignment, sum spectra were calibrated based on at least 4 glycopeptides per cluster with a signal/noise ratio of greater than 9. Spectra were excluded from further analysis when the total intensity of their analytes did not exceed 3 times the average intensity of the negative controls. Analytes were included in the final data analysis when their average signal/noise ratio (calculated per biological class) was greater than 9. This inclusion criteria resulted in extraction of 23 IgG_1, 12 $IgG_{2/3}$, and 10 IgG_4 glycoforms for all spectra. For further analysis of compositional features, derived traits reflecting groups of similar glycoforms were calculated as defined. For example, total galactose (G) was calculated as, (1/2*G1 + 2/2*G2) / [1*sum (IgG1 Glycan)].

Statistics

The ggplot2 R software package (version 3.1.2; RStudio, Boston, Mass) was used to graph age-dependent scatterplots, with locally weighted scatterplot smoothing illustrated as a blue line and the 95% CI indicated in the gray area. Glycan measurements were compared under the nonpaired nonequal variance and 2-sided hypothesis with a CI of 0.95. Calculated P values were adjusted by using the false discovery rate (Benjamini-Hochberg) method and reported as adjusted P values.[E10] Magnitude effect size is evaluated by using the Cliff delta method, with thresholds indicated as follows: $|d| < 0.147$ ("negligible"), $|d| < 0.33$ ("small"), $|d| < 0.474$ ("medium"), and $|d| \geq 0.474$ ("large"). Binomial and multinomial classification models were built using the Glmnet R package.[E11] To reduce the effect of age, each immunodeficient subject (with RRI or low IgA levels) was age matched to 3 healthy control subjects. Classifiers were trained by using either IgG LC-ESI-MS, UPLC glycan, or microsphere array data with the elastic net mixing parameter at 0.4. Coefficient weights of each measurement contributing to the model were evaluated. Predictive robustness was evaluated by using permutation tests.

REFERENCES

E1. Ercan A, Barnes MG, Hazen M, Tory H, Henderson L, Dedeoglu F, et al. Multiple juvenile idiopathic arthritis subtypes demonstrate pro-inflammatory IgG glycosylation. Arthritis Rheum 2012;64:3025-33.

E2. Driessen GJ, Dalm VA, van Hagen PM, Grashoff HA, Hartwig NG, van Rossum AM, et al. Common variable immunodeficiency and idiopathic primary hypogammaglobulinemia: two different conditions within the same disease spectrum. Haematologica 2013;98:1617-23.

E3. de Haan N, Reiding KR, Driessen G, van der Burg M, Wuhrer M. Changes in healthy human IgG Fc-glycosylation after birth and during early childhood. J Proteome Res 2016;15:1853-61.

E4. Falck D, Jansen BC, de Haan N, Wuhrer M. High-throughput analysis of IgG Fc glycopeptides by LC-MS. Methods Mol Biol 2017;1503:31-47.

E5. Jansen BC, Falck D, de Haan N, Hipgrave Ederveen AL, Razdorov G, Lauc G, et al. LaCyTools: a targeted liquid chromatography-mass spectrometry data processing package for relative quantitation of glycopeptides. J Proteome Res 2016;15:2198-210.

E6. Stockmann H, Duke RM, Millan Martin S, Rudd PM. Ultrahigh throughput, ultrafiltration-based n-glycomics platform for ultraperformance liquid chromatography (ULTRA(3)). Anal Chem 2015;87:8316-22.

E7. Stockmann H, Adamczyk B, Hayes J, Rudd PM. Automated, high-throughput IgG-antibody glycoprofiling platform. Anal Chem 2013;85:8841-9.

E8. Boesch AW, Brown EP, Cheng HD, Ofori MO, Normandin E, Nigrovic PA, et al. Highly parallel characterization of IgG Fc binding interactions. mAbs 2014;6:915-27.

E9. Brown EP, Licht AF, Dugast AS, Choi I, Bailey-Kellogg C, Alter G, et al. High-throughput, multiplexed IgG subclassing of antigen-specific antibodies from clinical samples. J Immunol Methods 2012;386:117-23.

E10. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc B 1995;57:289-300.

E11. Friedman J, Hastie T, Tibshirani R. Regularization paths for generalized linear models via coordinate descent. J Stat Software 2010;33:1-22.

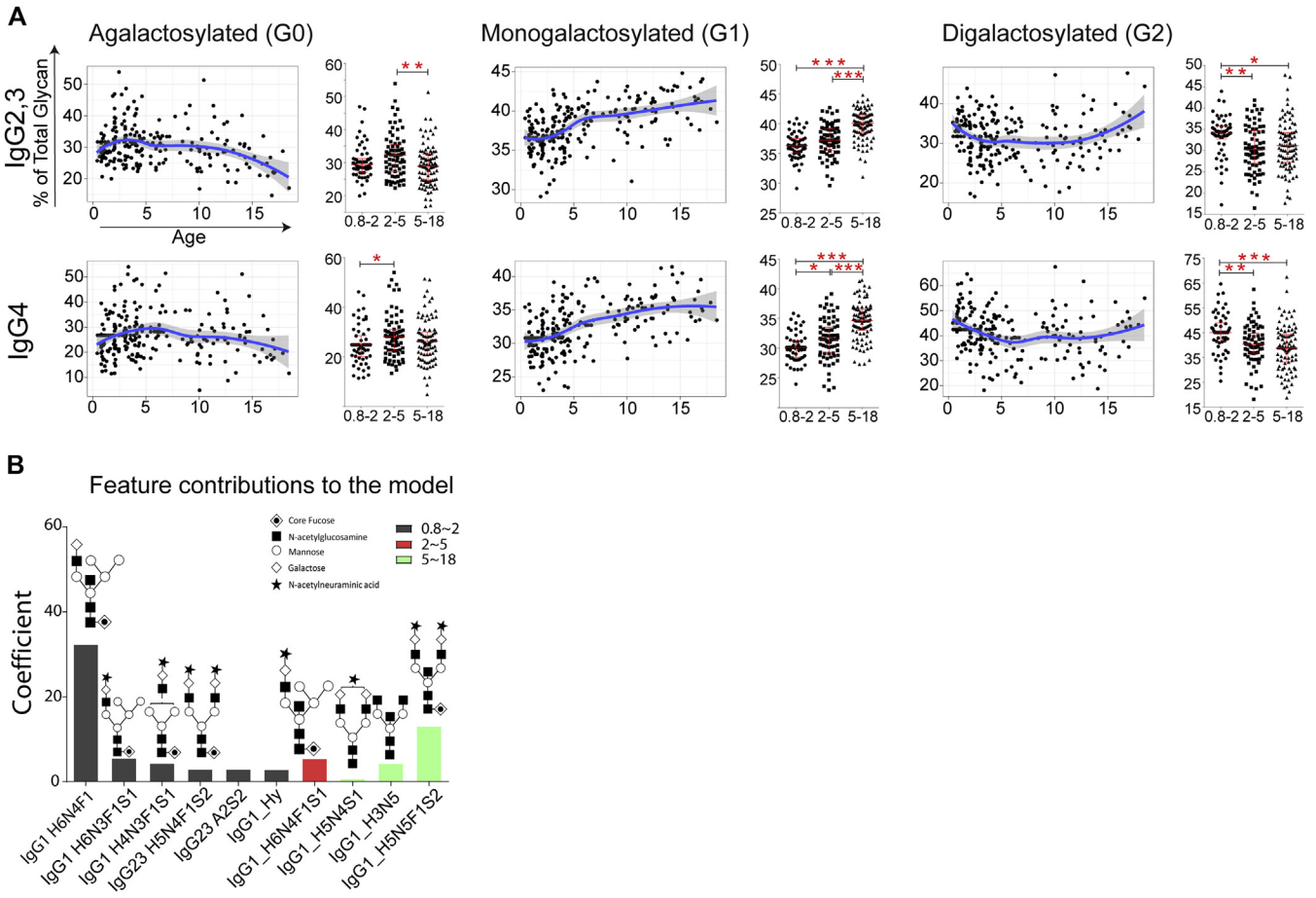

FIG E1. $IgG_{2/3}$ and IgG_4 Fc glycans in healthy children. **A,** Levels of galactosylated $IgG_{2/3}$ and IgG_4 glycans in 145 healthy children, as defined by using LC-ESI-MS, plotted by age. **B,** Glycan feature contributions to the age category classification model colored by the respective contribution to each age group. *P* values were adjusted in Fig E1, *A*, for multiple comparisons: $*P < .05$, $**P < .01$, and $***P < .001$.

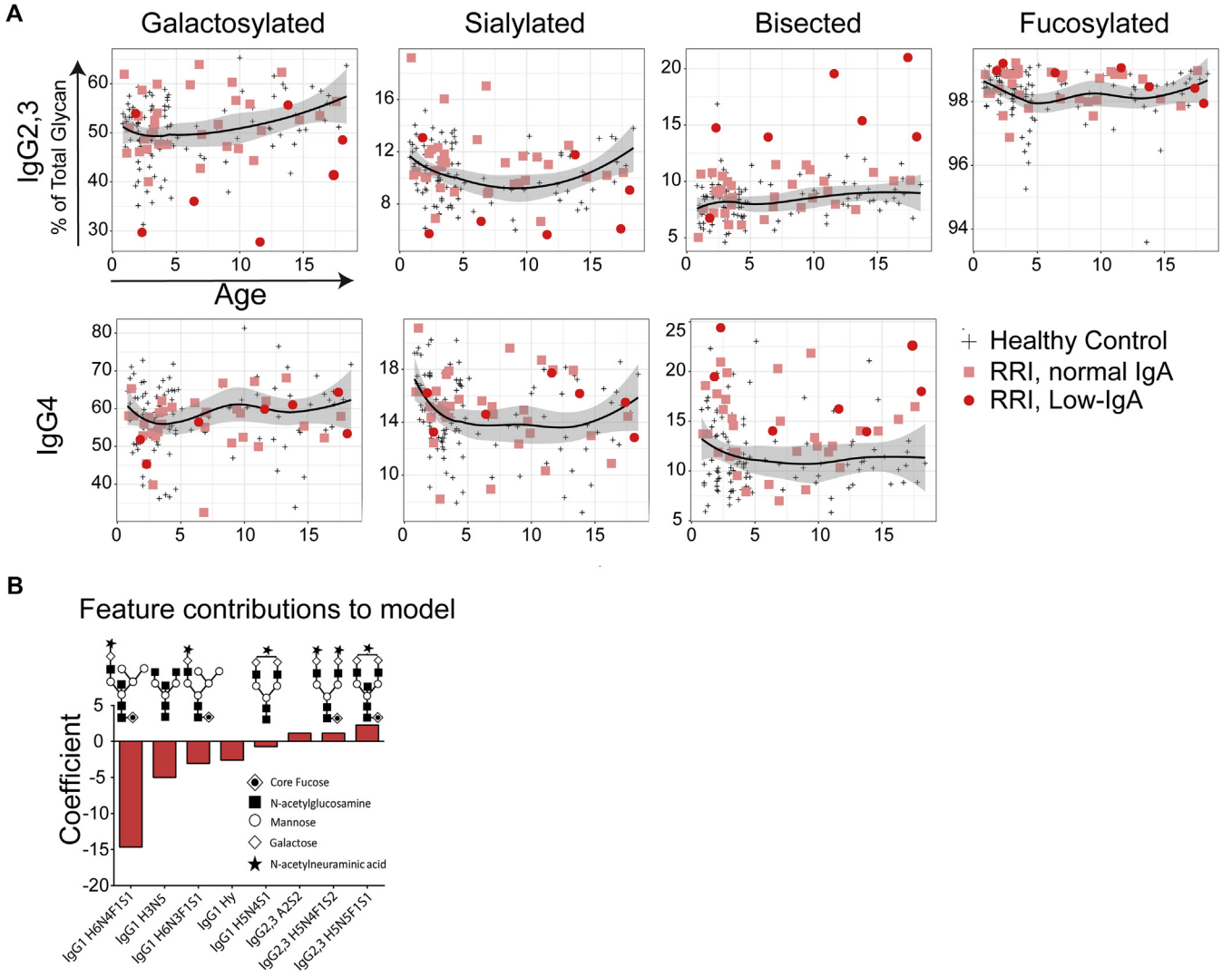

FIG E2. $IgG_{2/3}$ and IgG_4 Fc glycans in healthy children and immunodeficient subjects. **A,** Scatterplots of galactosylated, sialylated, bisected, fucosylated, and hybrid glycans by age in $IgG_{2/3}$ and IgG_4 levels from healthy children, patients with RRIs with normal IgA levels, and patients with RRIs with low IgA levels. *Black lines* indicate the locally weighted scatterplot smoothing line, and 95% CIs are indicated by the *gray shaded area*. **B,** Glycan feature contributions to the patient with RRI versus healthy control subject classification model.

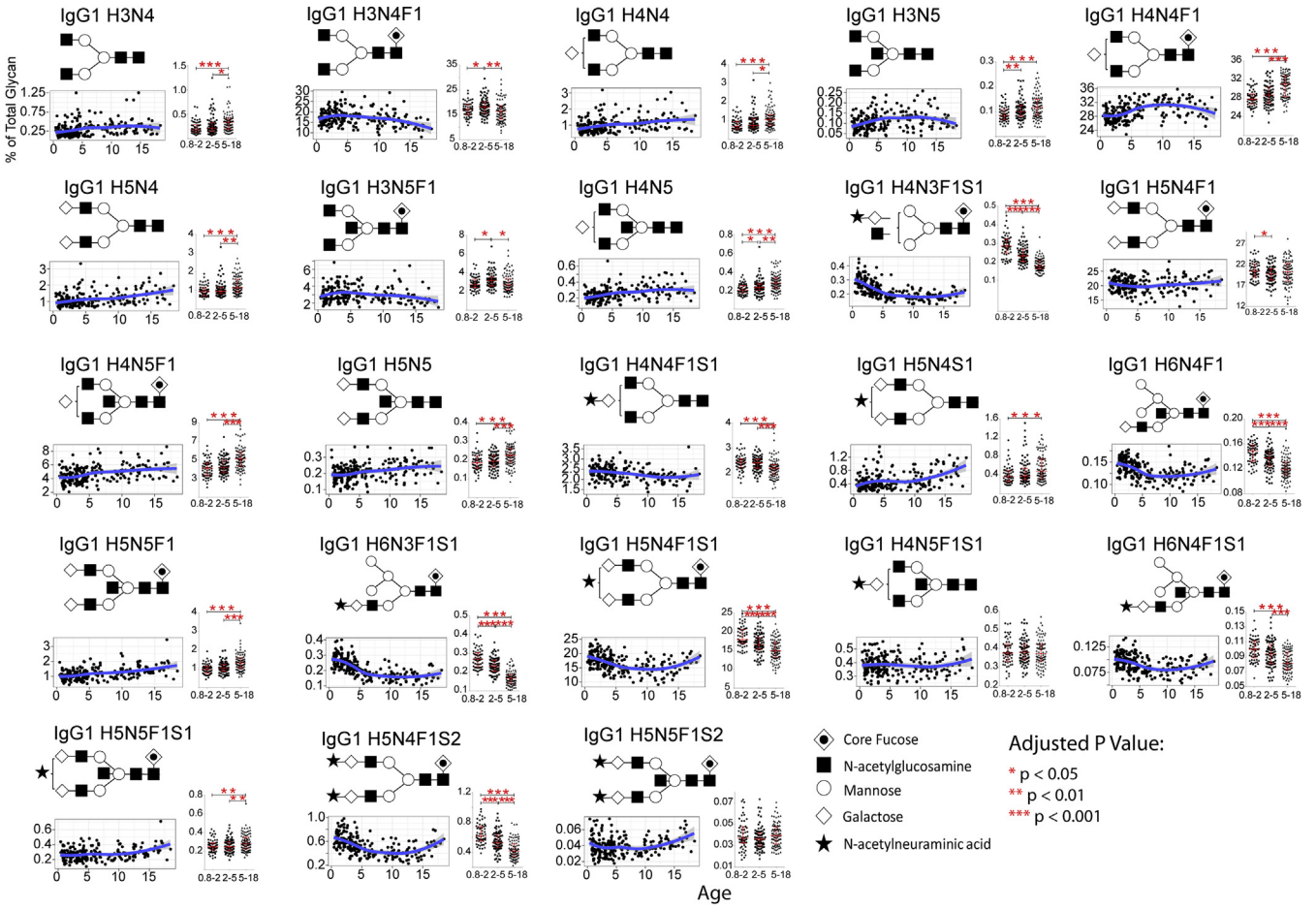

FIG E3. Individual IgG_1 Fc glycans in healthy children. Levels of individual IgG_1 Fc glycans in 145 healthy children, as defined by using LC-ESI-MS, plotted by age. *Left*, Scatterplots of glycan prevalence versus age. *Black lines* indicate the locally weighted scatterplot smoothing line, and 95% CIs are indicated by the *gray shaded area*. *P* values were adjusted for multiple comparisons: $*P < .05$, $**P < .01$, and $***P < .001$.

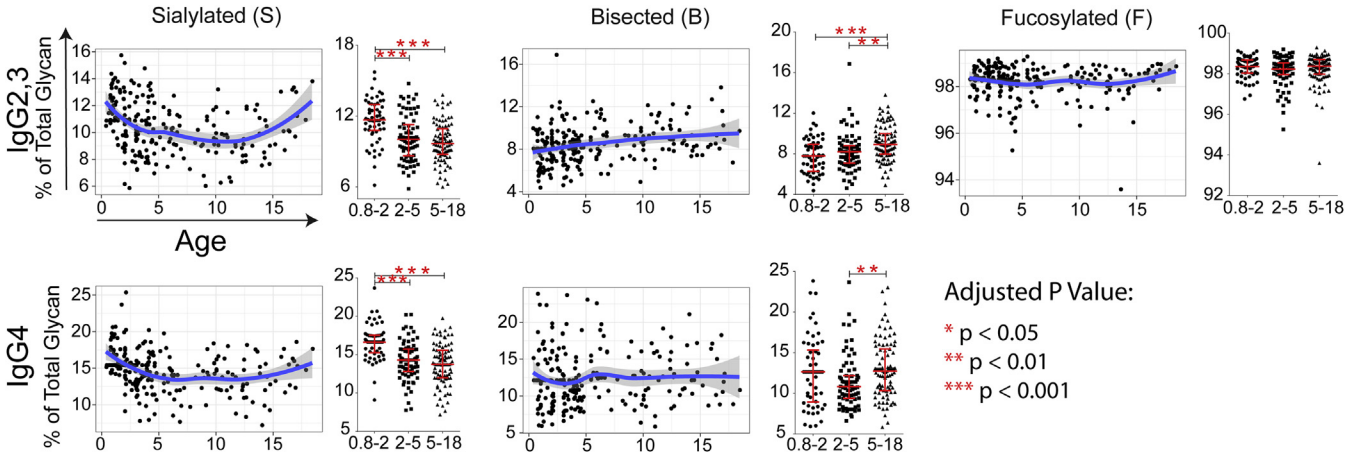

FIG E4. Summary $IgG_{2/3}$ and IgG_4 Fc glycans in healthy children. Level of sialylated, bisected, and fucosylated $IgG_{2/3}$ and IgG_4 Fc glycans in 145 healthy children, as defined by using LC-ESI-MS, plotted by age. *Black lines* indicate the locally weighted scatterplot smoothing line, and 95% CIs are indicated by the *gray shaded area*. *P* values were adjusted for multiple comparisons: **P* < .05, ***P* <.01, and ****P* < .001.

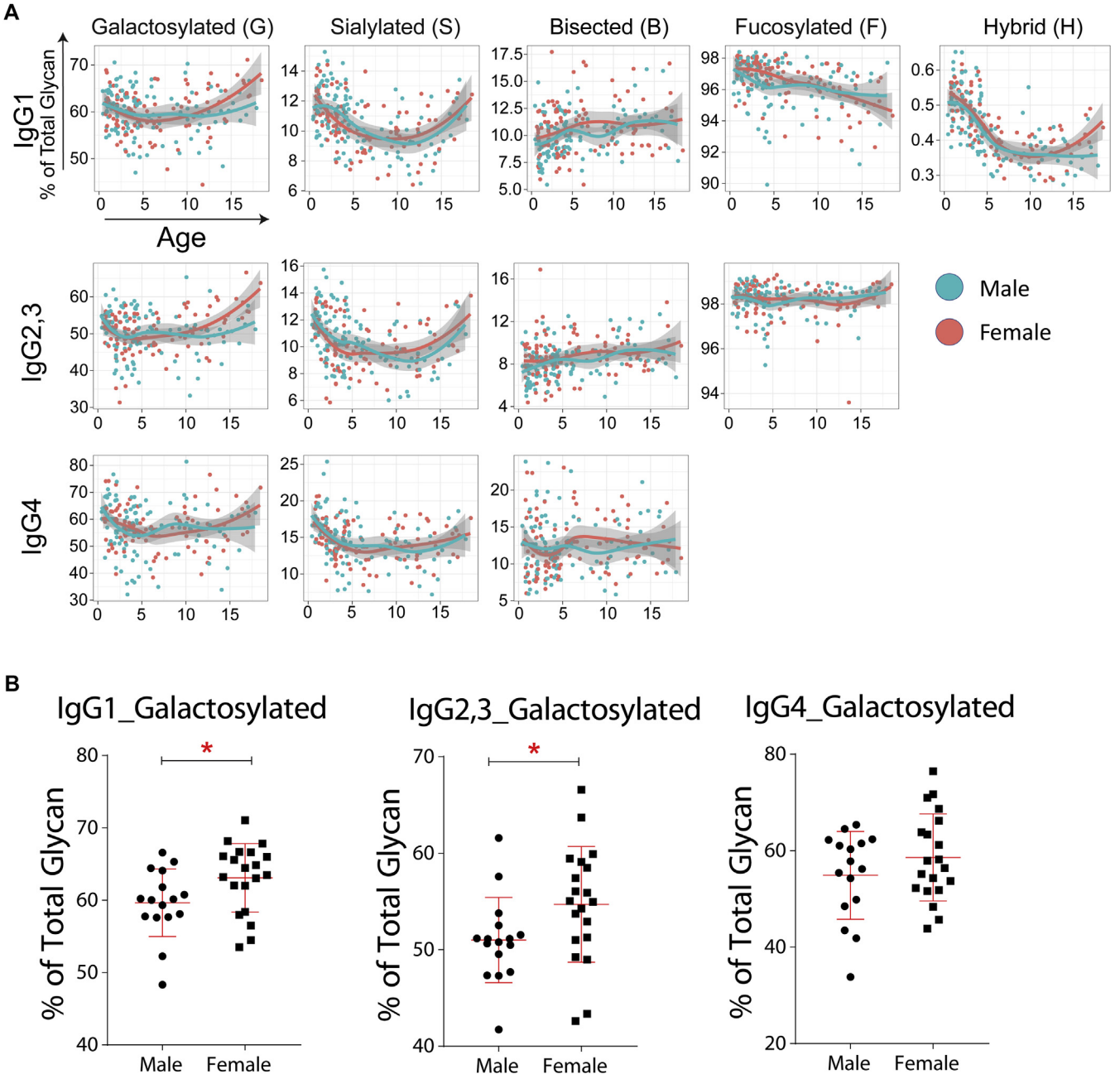

FIG E5. Summary IgG Fc glycans in healthy male versus female children. **A,** Levels of summary IgG_1 *(top)*, $IgG_{2/3}$ *(middle)*, and IgG_4 *(bottom)* glycans, including galactosylated, sialylated, bisected, fucosylated, and hybrid species, in healthy male (blue) and female (red) children, as defined by using LC-ESI MS, plotted by age. *Black lines* indicate the locally weighted scatterplot smoothing line, and 95% CIs are indicated by the *gray shaded area*. **B,** IgG galactosylation in male (n = 16) versus female (n = 20) subjects aged 12 years or older (mean ± SD): IgG_1, 60.0% ± 4.7% versus 63.1% ± 4.7% ($P = .035$); $IgG_{2/3}$, 51.0% ± 4.1% versus 54.7% ± 6.0% ($P = .047$); and IgG_4, 54.9% ± 9.1% versus 58.6% ± 9.0% ($P = .24$).

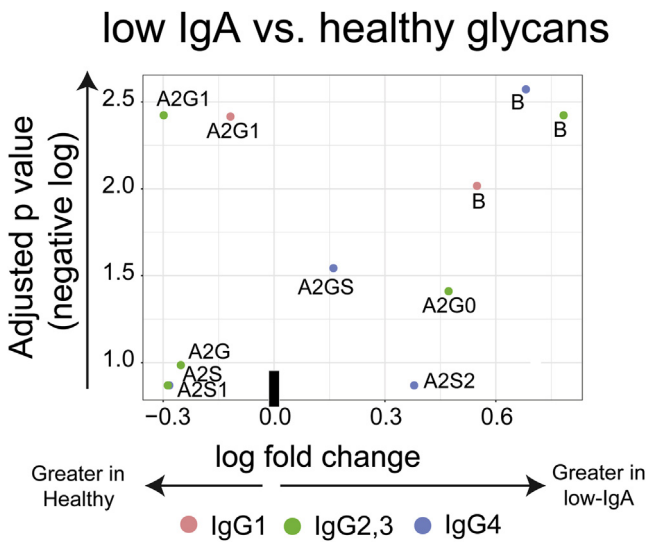

FIG E6. Comparison of IgG Fc glycans in healthy children versus children with low IgA levels. Volcano plot depicting differences between healthy subjects and patients with RRIs with low IgA levels. *A2*, Biantennary; *B*, bisected; *G*, galactosylation (0, 1, or 2); *Hy*, hybrid; *S*, sialylated (0, 1, or 2).

TABLE E1. Patients with RRIs

Age (y)	M/F	Infections	WBC (1000 cells/μL)	ALC (1000 cells/μL)	IgG (mg/dL)	IgA (mg/dL)	IgM (mg/dL)	IgE (U/mL)	IgG subclasses	IgG_1 (mg/dL)	IgG_2 (mg/dL)	IgG_3 (mg/dL)	IgG_4 (mg/dL)	Pneumococcal titer	CH50 (60-144)
0.9	M	Rec otitis	14.89	8.79	512	67	74	1	Low IgG_4	330	70	114 H	<1 L	Good	Normal
1.0	M	Rec sinusitis	7.25	5.18	469	36	59		Low IgG_4	322	53	74	<1 L	Good	Not done
1.1	M	Rec otitis, rec sinusitis	8.28	4.65	390	47	76	26	Normal	256	52	26	2	Not done	Normal
1.8	M	Rec otitis	11.41	3.65	481	66	87		Low IgG_4	295	113	85	<1 L	Good	156 H
2.1	M	Rec otitis, rec pneumonia, rec sinusitis	7.73	3.32	617	79	46	14	Normal	392	99	30	2	Good	57 L
2.3	F	Rec ear otitis, rec pneumonia	10.14	4.85	649	65	88	18	Normal	412	81	42	7	Good	Not done
2.6	F	Rec otitis, rec pneumonia	18.59	8.88	468	41	64	4	Normal	340	63	35	2	Good	Not done
2.6	F	Rec otitis, rec pneumonia	17.35	8.88	527	63	57	4	Normal	363	56	27	6	Good	Not done
2.8	M	Rec otitis	5.27	1.94	665	81	37	52	Normal	459	51	22	14	Low	Not done
3.0	M	Rec sinusitis	8.44	6.08	746	180	48	12	High IgG_3	516	134	83 H	17	Good	Normal
3.1	M	Rec pneumonia	13.19	4.91	733	61	82	13	Normal	509	83	24	2	Good	Not done
3.2	M	Rec otitis	9.86	4.28	567	49	52	21	Normal	377	89	36	4	Good	Normal
3.4	M	Rec otitis	9.84	4.1	881	110	107	6	Normal	525	114	61	9	Not done	Not done
3.6	M	Rec sinusitis	9.23	4.85	1171	78	89	3	High IgG_3	762	133	130 H	24	Good	Not done
4.3	F	Rec otitis, rec pneumonia	5.58	2.42	848	75	108	153	Normal	573	159	44	1	Good	Not done
4.3	M	Rec otitis			680	91	54	14	Normal	441	88	33	8	Good	Not done
4.3	M	Rec otitis, rec pneumonia	5.85	2.52	1188	172	147	76	High IgG_4	720	207	43	125 H	Good	Normal
5.5	M	Rec otitis, rec pneumonia, rec sinusitis	6.11	3.2	898	79	60	9	Normal	575	215	22	36	Good	Not done
5.9	M	Rec pneumonia	6.3	2.86	705	125	69	108	Normal	384	205	27	7	Good	Not done
6.0	F	Rec sinusitis	5.61	2.7	1142	132	153	145	Normal	705	302	47	30	Good	Normal
6.1	F	Rec pneumonia	10.47	4.46	791	124	96	715 H	Normal	422	272	81	64	Good	Normal
6.8	M	Rec otitis	6.71	3.03	675	186	61	90	Normal	357	212	64	96	Not done	Not done
7.0	F	Rec otitis	6.43	3.57	667	82	110	21	Normal	462	107	44	13	Good	Not done
9.1	M	Rec sinusitis, rec pneumonia	9.58	2.32	779	91	57	42	Normal	429	196	68	70	Low	Normal
9.5	M	Rec otitis, rec pneumonia, rec sinusitis	5.33	2.14	764	112	87	25	Normal	519	143	33	11	Good	Normal
9.5	M	Rec sinusitis	9.7	2.84	927	96	119	11	Normal	482	236	156	34	Good	Normal
10.0	F	Rec sinusitis	7.89	2.35	1139	128	166	51	High IgG_3	700	277	131 H	17	Good	Normal
10.7	M	Rec sinusitis	7.34	3.54	711	154	113	125	Normal	401	212	67	10	Good	Normal
11.1	M	Rec otitis, rec sinusitis	6.51	1.17	1023	306	99	145	Normal	477	357	72	26	Good	Normal
13.2	M	Rec pneumonia, rec sinusitis			866			9	Normal	386	282	76	11	Good	Not done
14.7	F	Rec otitis	6.92	2.41	857	64	246	13	Normal	496	178	42	12	Not done	56L
16.3	M	Rec sinusitis	6.97	2.12	1104	149	173	311 H	High IgG_4	640	256	80	111 H	Good	Normal
17.7	M	Rec sinusitis, rec pneumonia	5.7	1.77	965	149	88	317 H	Normal	534	264	24	13	Good	Not done

ALC, Absolute lymphocyte count; *CH50*, 50% hemolytic complement; *F*, female; *H*, high; *L*, low; *M*, male; *rec*, recurrent; *WBC*, white blood cell count.

TABLE E2. Subjects with low IgA levels

Subject	1	2	3	4	5	6	7
Age (y)	1.7	2.2	6.3	9.8	11.5	13.7	17.3
M/F	M	F	F	F	M	F	F
Infections	Rec otitis	Rec otitis	Rec otitis	Rec otitis, rec sinusitis	Rec otitis, rec pneumonia, rec sinusitis	Rec sinusitis	Rec sinusitis
WBC (1000 cells/μL)	11.52	6.56	9.9	6.94	5.35	5.6	6.3
ALC (1000 cells/μL)	4.69	3.74	4.67	3.29	1.69	2.39	1.97
IgG (mg/dL)	432	524	1096	894	1115	693	1204
IgA (mg/dL)	13	10	<7	54	<7	53	<7
IgM (mg/dL)	Not done	64	130	164	185	114	131
IgE (U/mL)	56 H	Not done	42	30	49	3	4
IgG subclasses	Normal	Normal	Normal	Normal	Normal	Normal	Normal
IgG_1 (mg/dL)	309	367	633	532	650	385	745
IgG_2 (mg/dL)	52	74	367	259	369	209	354
IgG_3 (mg/dL)	42	16	24	78	35	35	37
IgG_4 (mg/dL)	1	1	8	31	12	40	10
Pneumococcal titer	Low	Good	Good	Good	Good	Good	Good
CH50 (60-144)	45 L	Not done	Not done	Not done	Not done	Normal	Not done

ALC, Absolute lymphocyte count; *CH50*, 50% hemolytic complement; *F*, female; *H*, high; *L*, low; *M*, male; *rec*, recurrent; *WBC*, white blood cell count.