# IgG1 Fc N-glycan galactosylation as a biomarker for immune activation

Sanne E. de Jong[1], Maurice H.J. Selman[2], Ayola A. Adegnika[1,3,4], Abena S. Amoah[1,5], Elly van Riet[1], Yvonne C.M. Kruize[1], John G. Raynes[6], Alejandro Rodriguez[7], Daniel Boakye[5], Erika von Mutius[8,9], André C. Knulst[10], Jon Genuneit[11], Philip J. Cooper[7,12], Cornelis H. Hokke[13], Manfred Wuhrer[2], Maria Yazdanbakhsh[1],*

[1]Leiden Immunoparasitology Group, Department of Parasitology, Leiden University Medical Center, Leiden, the Netherlands, [2]Center for Proteomics and Metabolomics, Leiden University Medical Center, Leiden, the Netherlands, [3]Center of Medical Research Lambaréné (CERMEL), Lambaréné, Gabon, [4]Institute for Tropical Medicine, University of Tübingen, Tübingen, Germany, [5]Department of Parasitology, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Accra, Ghana, [6]Department of Immunology and Infection, London School of Hygiene and Tropical Medicine, London, United Kingdom, [7]Instituto de Microbiologia, Universidad San Francisco de Quito, Quito, Ecuador, [8]Dr von Hauner Children's Hospital, Ludwig Maximilian University, Munich, Germany, [9]Comprehensive Pneumology Center Munich (CPC-M), Member of German Center for Lung Research (DZL), Munich, Germany, [10]Department of Dermatology/Allergology, University Medical Center Utrecht, Utrecht, the Netherlands, [11]Institute of Epidemiology and Medical Biometry, Ulm University, Ulm, Germany, [12]Institute of Infection and Immunity, St George's University of London, London, United Kingdom, [13]Leiden Parasite Glycobiology Group, Department of Parasitology, Leiden University Medical Center, Leiden, the Netherlands.

*Address correspondence to M.Y. (m.yazdanbakhsh@lumc.nl).

Supplementary information
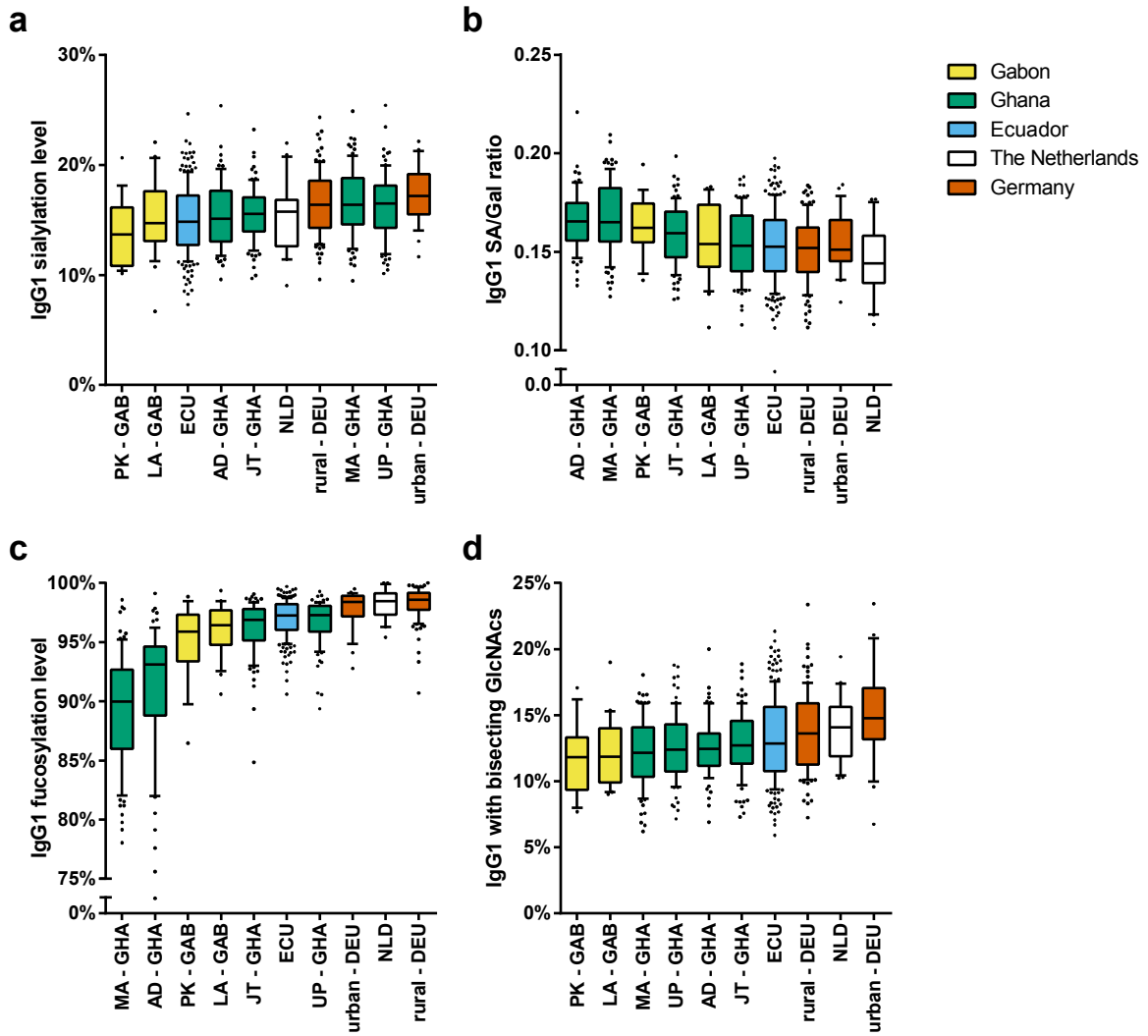
Supplementary Figure S1. a) Sialylation, b) sialic acids per galactose, c) fucosylation and d) abundance of bisecting GlcNAcs of IgG1 compared between different communities and countries. Boxplots with 10-90% whiskers.
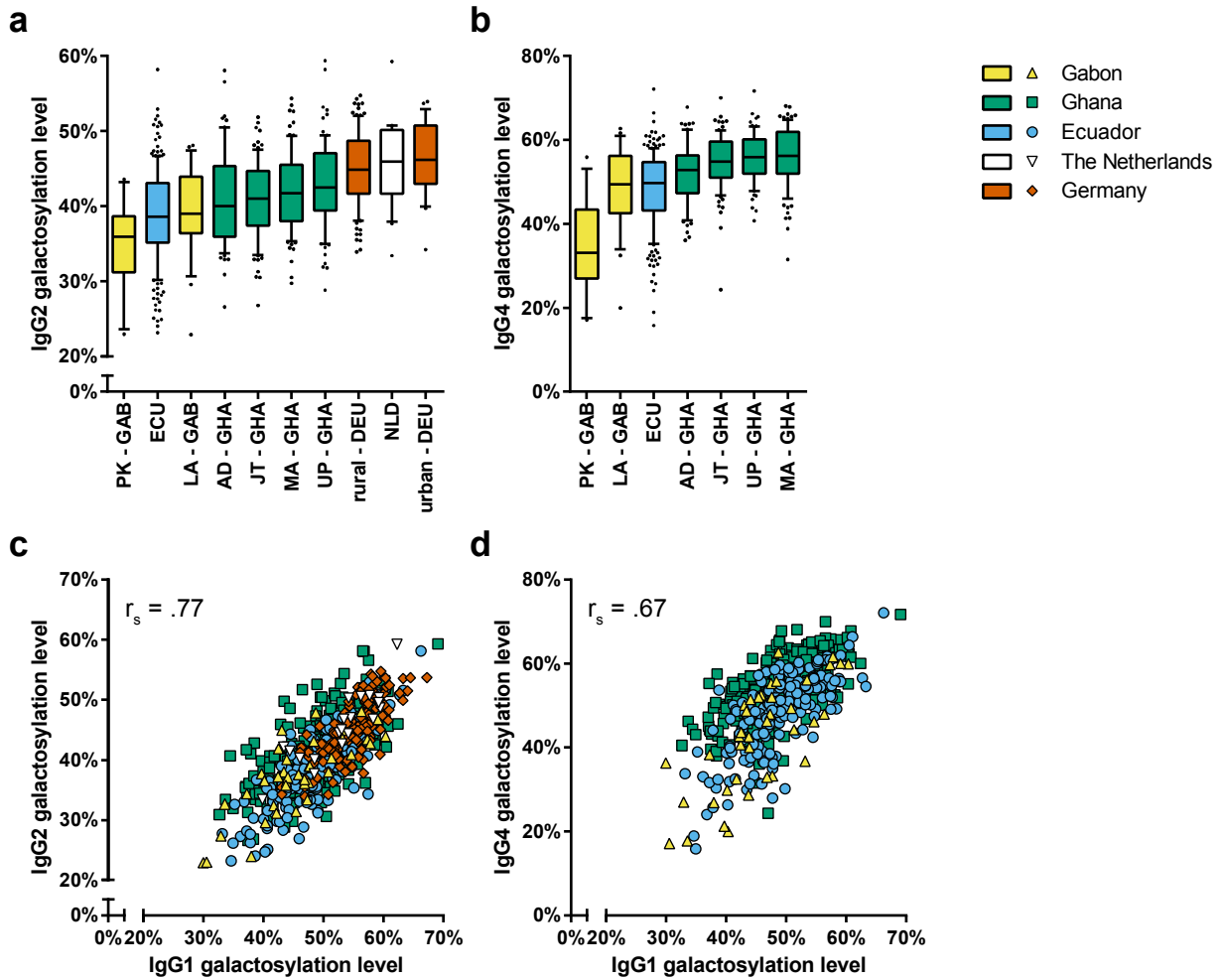
Supplementary Figure S2. a) IgG2 galactosylation compared between various communities and countries. b) IgG4 galactosylation compared between populations. Germany and the Netherlands were left out of the analysis, as the IgG4 levels of many children was below detection limit. c) Spearman's correlation between IgG1 galactosylation and IgG2 galactosylation (n=700). d) Spearman's correlation between IgG1 galactosylation and IgG4 galactosylation (n=555). Boxplots have 10-90% whiskers. Spearman's rho correlation coefficient r_s is shown, with $P < .001$.

a

b
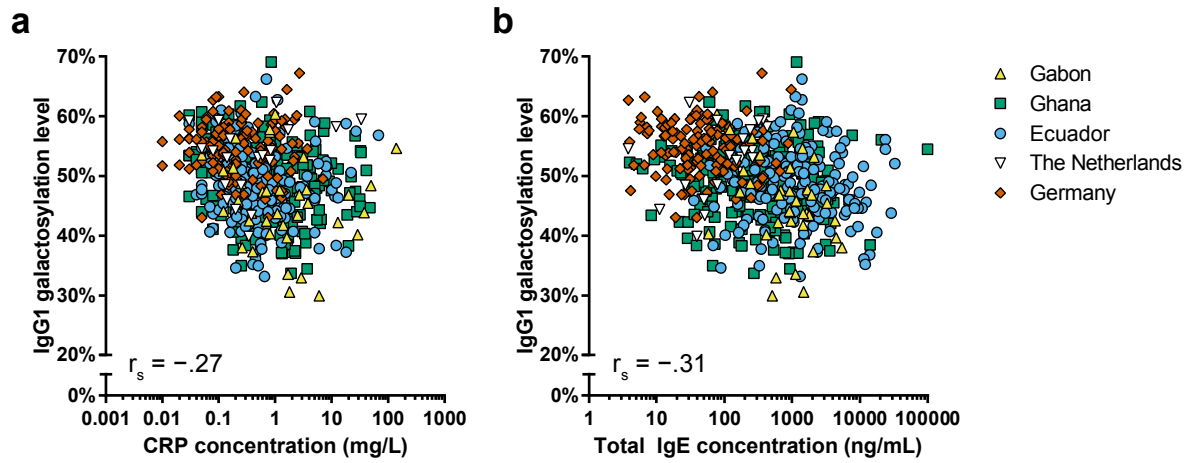
Supplementary Figure S3. Spearman's correlation between IgG1 galactosylation and a) CRP levels and b) total IgE levels. Spearman's rho correlation coefficient r_s is shown, with $P < .001$ (n=700).

Supplementary Table S1. Comparison of IgG1 galactosylation between all countries, between all communities and between communities within a country with the non-parametric Kruskal-Wallis H test and Mann Whitney U test. IgG1 galactosylation differed significantly between the various countries and communities. When comparing communities within one country only, the Gabonese communities differed significantly from each other, as did the Ghanaian communities, while the Ecuadorian and German communities did not. Post-hoc testing results are shown in Supplementary Table S3 and Table S4.

Comparison of IgG1 galactosylation	Total number of children	Test statistics	P value
Between the 5 countries	700	H(4) = 112.08	<.001
Between the 16 communities of all countries	700	H(15) = 163.59	<.001
Between the 2 Gabonese communities	39	U = 91.00, z = −2.66	.007
Between the 4 Ghanaian communities	323	H(3) = 39.50	<.001
Between the 7 Ecuadorian communities	193	H(6) = 10.24	.111
Between the 2 healthy German communities	125	U = 988.00, z = −1.62	.107

Supplementary Table S2. P values resulting from Dunn-Bonferroni post-hoc tests for comparison between all countries as in Supplementary Table S2. IgG1 galactosylation differed significantly between Gabon and Ghana, Gabon and Germany, Gabon and the Netherlands, Ghana and Germany, Ecuador and Germany, and Ecuador and the Netherlands.

Country	P value post-hoc tests for comparisons between countries				
	Gabon	Ghana	Ecuador	Germany	The Netherlands
Gabon		.034	.276	<.001	.001
Ghana	.034		1.000	<.001	.099
Ecuador	.276	1.000		<.001	.027
Germany	<.001	<.001	<.001		1.000
The Netherlands	.001	.099	.027	1.000	

Table S3. P values resulting from Dunn-Bonferroni post-hoc tests for comparison of communities within Ghana as in Supplementary Table S3. IgG1 galactosylation differs significantly between the Ghanaian communities AD, MA and JT as compared to UP.

Community	P value post-hoc tests for comparison between communities			
	GHA, AD	GHA, MA	GHA, JT	GHA, UP
GHA, AD		.052	.222	<.001
GHA, MA	.052		1.000	.001
GHA, JT	.222	1.000		<.001
GHA, UP	<.001	.001	<.001	

Supplementary Table S4. Spearman's correlation coefficient r_s between various IgG1 glycan modifications, with accompanying P values.

Modification	Galactosylation		Sialylation		SA/Gal		Fucosylation		Bisecting GlcNAc	
	r_s	P value	r_s	P value	r_s	P value	r_s	P value	r_s	P value
Galactosylation			.81	<.001	.29	<.001	.16	<.001	−.03	.497
Sialylation	.81	<.001			.77	<.001	−.03	.500	−.07	.051
SA/Gal	.29	<.001	.77	<.001			−.21	<.001	−.10	.012
Fucosylation	.16	<.001	−.03	.500	−.21	<.001			.12	.002
Bisecting GlcNAc	−.03	.497	−.07	.051	−.10	.012	.12	.002		

Supplementary Table S5. Spearman's correlation coefficient r_s of various glycan modifications between IgG1, IgG2 and IgG4 subclasses, with accompanying P values. Germany and the Netherlands were left out of the analysis with IgG4, as the IgG4 levels of many children was below detection limit.

Modification	IgG1 with IgG2		IgG1 with IgG 4		IgG2 with IgG 4	
	r_s	P value	r_s	P value	r_s	P value
Galactosylation	.77	<.001	.67	<.001	.63	<.001
Sialylation	.75	<.001	.75	<.001	.71	<.001
SA/Gal	.80	<.001	.75	<.001	.62	<.001
Fucosylation	.36	<.001				
Bisecting GlcNAc	.78	<.001	.74	<.001	.66	<.001

Supplementary Table S6. Definitions and descriptive characteristics of the infrastructure, socioeconomic and lifestyle indicators of the Ecuadorian population, as determined by Rodriquez *et al*[12].

	Indicators	Definition	Categories
Infrastructure	Administrative grade	Political/administrative division	Towns, parish
	Transport access	Type of access used to arrive at communities	River, road
	Electrical grid	Presence of a connection to the electrical grid	No, yes
	Piped water system	Presence of a piped water system (untreated water only)	No, yes
	Telephone system	Access to the national telephone network	No, yes
	Health centre	Presence of a health centre	No, yes
	Pharmacy	Presence of a pharmacy	No, yes
	Secondary school	Presence of secondary schools	No, yes
	Shops	Number of shops (commercial infrastructure)	
socioeconomics	Father's education	% of households in which the father has a secondary education	
	Mother's education	% of households in which the mother has a secondary education	
	Household income	% of households with an income ≥ US$150/month	
	Access to electricity	% of households with electricity (electrical grid or generator)	
	Material goods	% of households with (all of) refrigerator, TV and stereo system	
	Cement house	% of households with cement walls	
	Gas for cooking	% of households that use propane gas for cooking	
	Motor vehicles	% of households with motor vehicles (boat or car)	
	Crowded household	% of households that are crowded (>3 persons per bedroom)	
Lifestyle	Farming activities	% of households that work in agricultural activities	
	Cat in house	% of households that have a cat living inside the house	
	Dog in house	% of households that have a dog living inside the house	
	TV in house	% of households that have a television	
	TV viewing	% of study children who watch television >1 h daily	
	Hamburger consumption	% of study children who consume ≥ 1 hamburger per month	
	Fizzy drinks consumption	% of study children who consume fizzy drinks daily	
	Physical exercise	% of study population with daily physical activity (not sedentary)	
	Migration	% of study children who has lived for ≥3 months outside the study area in the past	
	STH infection rate	% of study population with intestinal helminth *Ascaris lumbricoides, Trichuris trichiura, Strongyloides stercoralis*	

Supplementary Table S7. Theoretical m/z values of human IgG Fc glycopeptides detected.

Glycan species[a]	IgG1 P01857[b] $E_{293}EQYNSTYR_{301}$[c]		IgG2 P01859[b] $E_{293}EQFNSTFR_{301}$[c]		IgG4 P018561[b] $E_{293}EQFNSTYR_{301}$[c]	
	$[M + 2H]^{2+}$	$[M + 3H]^{3+}$	$[M + 2H]^{2+}$	$[M + 3H]^{3+}$	$[M + 2H]^{2+}$	$[M + 3H]^{3+}$
No	595.260	397.176	579.265	386.513	587.262	391.844
G0F	1317.527	878.687	1301.532	868.024	1309.529	873.356[d1]
G1F	1398.553	932.705	1382.558	922.042	1390.556	927.373[d2]
G2F	1479.580	986.722	1463.585	976.059	1471.582	981.391
G0FN	1419.067	946.380	1403.072	935.717	1411.069	941.049[d3]
G1FN	1500.093	1000.398	1484.098	989.735	1492.096	995.066[d4]
G2FN	1581.119	1054.416	1565.124	1043.752	1573.122	1049.084
G1FS	1544.101	1029.737	1528.106	1019.073	1536.104	1024.405[d5]
G2FS	1625.127	1083.754	1609.132	1073.091	1617.130	1078.423
G1FNS	1645.641	1097.430	1629.646	1086.766	1637.643	1092.098
G2FNS	1726.667	1151.447	1710.672	1140.784	1718.670	1146.116
G0	1244.498	830.001	1228.503	819.338	-	-
G1	1325.524	884.019	1309.529	873.356[d1]	-	-
G2	1406.551	938.036	1390.556	927.373[d2]	-	-
G0N	1346.038	897.694	1330.043	887.031	-	-
G1N	1427.064	951.712	1411.069	941.049[d3]	-	-
G2N	1508.090	1005.730	1492.096	995.066[d4]	-	-
G1S	1471.072	981.051	1455.077	970.387	-	-
G2S	1552.098	1035.068	1536.104	1024.405[d5]	-	-
G1NS	1572.612	1048.744	1556.617	1038.081	-	-
G2NS	1653.638	1102.761	1637.643	1092.098	-	-

[a]Glycan structural features are given in terms of number of galactoses (G0, G1, G2), fucose (F), bisecting *N*-acetylglucosamine (N), and *N*-acetylneuraminic acid (S). [b]SwissProt entry number. [c]Tryptic IgG glycopeptide sequence. [d]Isomeric glycopeptide species of IgG2 and IgG4.