

Letter to the Editor

Genetic variation in IL-10 is associated with atopic reactivity in Gabonese school children

To the Editor:

Studies investigating the immunogenetics of asthma and/or atopy have so far only involved populations residing in the developed world. Given that populations can be genetically diverse, environmental conditions different, and causal pathways of allergic diseases not the same, it is of interest to establish whether genetic associations found in the developed world are also true in the developing world, where the frequency of allergic diseases is low but increasing.¹ The human *IL10* gene has been described to predict asthma or atopy phenotypes in western populations,² but may have evolved differently in populations in which adequate IL-10 responses are important to prevent inflammation from sustained challenges by infectious pathogens. Because we previously found that parasite-induced IL-10 responses protected Gabonese schoolchildren from atopic reactivity,³ we postulated that variations in the human *IL10* gene that promote high IL-10 levels would be associated with reduced atopy in populations residing in Africa.

We genotyped 100 Gabonese schoolchildren (5-14 years old; 53 boys and 47 girls) with known skin prick test (SPT) reactions to house dust mite (HDM; 25 positive and 75 negative responders) for 8 biallelic single nucleotide polymorphisms (SNPs; -597AC, -824CT, -1087AG, -1354AG, -2736AC, -3538AT, -6208CG, -6752AT) and 1 insertion/deletion polymorphism (-7400indel) in the 5'-flanking region of the human *IL10* gene and investigated

associations with SPT reactivity and phytohemagglutinin (PHA)-induced IL-10 levels for individual polymorphisms and phased haplotypes.⁴

Allele and genotype frequencies for the total study population and HDM-SPT negative and positive children are presented in this article's Table E1 and associations with *in vitro* IL-10 production in Table E2 in the Online Repository at www.jacionline.org. The polymorphic -6208C(→G), -3538A(→T), -2736A(→C), -1354A(→G), and -1087G(→A) alleles all occurred more frequently in the HDM-SPT positive group than in the negative group, and in logistic regression (adjusted for age and sex), a significant 2-fold increased HDM-SPT risk was found for -2736A(→C) (odds ratio [OR], 2.03; 95% CI, 1.04-3.95; $P = .037$) and -1354A(→G) (OR, 1.97; 95% CI, 1.01-3.82; $P = .045$; see this article's Table E1 in the Online Repository at www.jacionline.org). The -2736A(→C) allele was significantly associated with lower IL-10 production in the total study population ($P = .014$, without Bonferroni correction); similar associations were found when HDM-negative and HDM-positive children were studied separately, although differences were not statistically significant. For each of the 9 polymorphisms, the allele associated with a higher risk for HDM-SPT was also associated with a lower capacity to produce IL-10.

We next studied associations with well described proximal (-1087AG/-824CT/-597AC) and distal (positions -3538AT/-2736AC) haplotype blocks in this promoter gene region: in line with our hypothesis that high IL-10 levels protect against atopic reactivity, the proximal ATA and distal TC were both significantly associated with lower

TABLE I. Haplotype analysis for *IL10* polymorphisms in relation to atopic reactivity and IL-10 production*

Haplotypes	Frequencies			Atopic reactivity		IL-10 production	
	Total	SPT ^{neg}	SPT ^{pos}	OR (95% CI)	<i>P</i> value	$\beta \pm SE$	<i>P</i> value
Proximal							
GCC	0.40	0.36	0.52	1 (Ref)	.040	Ref	.014
ATA	0.39	0.42	0.28	0.40 (0.17-0.95)	.296	0.267 $\pm$ 0.106	.219
ACC	0.22	0.22	0.20	0.60 (0.23-1.56)		0.151 $\pm$ 0.122	
Distal							
TC	0.54	0.58	0.42	1 (Ref)	.024	Ref	.009
AA	0.28	0.24	0.40	2.79 (1.16-6.68)	.182	-0.290 $\pm$ 0.109	.035
TA	0.18	0.18	0.18	2.00 (0.73-5.52)		-0.272 $\pm$ 0.127	
Extended							
TC-G-ATA	0.39	0.42	0.28	1 (Ref) [†]	.011	Ref [†]	.037
AA-A-GCC	0.21	0.15	0.38	3.74 (1.38-10.13)		-0.282 $\pm$ 0.125	
All SNPs							
in - AG-TC-G-ATA	0.37	0.40	0.26	1 (Ref) [†]	.050	Ref [†]	.205
in - TC-AA-A-GCC	0.11	0.09	0.18	3.55 (1.02-12.34)	.029	-0.213 $\pm$ 0.167	.029
del - TC-AA-A-GCC	0.09	0.06	0.18	4.50 (1.19-16.98)		-0.380 $\pm$ 0.172	

*Haplotype combinations include proximal, -1087AG/-824CT/-597AC; distal, -3538AT/-2736AC; extended, -3538AG/-2736CT/-1354AG/-1087AG/-824CT/-597AC; and all SNPs, -7400indel/-6752AT/-6208CG/-3538AG/-2736CT/-1354AG/-1087AG/-824CT/-597AC. Atopic reactivity was defined as a positive SPT to HDM. IL-10 production was measured by *in vitro* stimulation of PBMCs with PHA. Associations between (major) haplotypes and HDM-SPT and (log-transformed) IL-10 levels were studied by using multiple logistic and linear regression models, while adjusting for age, sex, and [†]minor haplotypes. Compared with the reference (the most frequent haplotype in a model), an OR >1 and OR <1 indicate an increased and reduced (atopy) risk, respectively, and a negative and a positive linear regression coefficient (β) a lower and higher IL-10-PHA production.

HDM-SPT risk and higher IL-10 levels compared with GCC and AA, respectively (Table I). When we analyzed haplotype combinations that included the proximal and distal haplotypes and the in-between polymorphism at position -1354 (-3538AT/-2736AC/-1354AG/-1087AG/-824CT/-597AC), the HDM-SPT protective proximal ATA was found to occur only in the combination TC-G-ATA, and GCC in any but predominantly the AA-A-GCC combination. In this extended combination, the odds ratio for skin test reactivity increased from 2.50, as found for GCC versus ATA, to 3.74 for AA-A-GCC versus TC-G-ATA. Again, IL-10-PHA levels were significantly lower in AA-A-GCC versus TC-G-ATA (Table I). Haplotype analysis including all 9 polymorphisms did not change the associations as described.

This is the first evidence that variation in the *IL10* gene that promotes high IL-10 responses is associated with reduced risk for atopic reactivity in children living in Africa. Although our primary aim was to demonstrate this association and not to identify specific functional mutations, our findings indicate quite clearly that the distal polymorphisms (AA-A vs TC-G) are more informative than the proximal ones. This is in line with Gibson et al.,⁵ who, like us but in contrast with others,^{6,7} reported that (AA-A)-GCC was associated with significantly lower IL-10-PHA levels than (TC-G)-ATA.

Interestingly, from literature it appears that the proximal GCC, which is associated with low IL-10 production and increased HDM-SPT risk in our study population, occurs at a higher frequency in white subjects (49% to 58%) than in African subjects (31% to 38%), and the other way around for the HDM-SPT protective proximal ATA (34% to 39% in African subjects and 18% to 25% in white subjects).^{4,8,9} We believe that this distinct distribution pattern between geographically diverse populations supports an evolutionary role for the *IL10* gene: whereas a balanced frequency of low and high IL-10 promoting genotypes in African populations indicates their equal beneficence in environments of high infectious exposures (low IL-10 responses facilitate pathogen clearance, but high IL-10 responses prevent immunopathology), the excessive occurrence of a low IL-10 promoting genotype in white populations underscores the redundancy of high IL-10 responses in populations living under affluent conditions.

Our current finding that variations in the *IL10* gene that promote high IL-10 production are associated with lower atopy risk adds a new dimension to our previous finding that parasite-induced IL-10 production reduces the risk for skin test reactivity in Gabonese children,³ namely that in populations continuously exposed to infectious pathogens, the IL-10-mediated risk for atopy is regulated at both an environmental (infection) and a genetic level. Considering the progressive urbanization of the developing world, it is therefore of great interest to establish the role that genetic versus environmental mechanisms play in the activation of IL-10 responses and consequent risk for allergies and other inflammatory diseases in populations in transition.

Anita H. J. van den Biggelaar, PhD^{a,b,e}

Thanh-Duc Hua^c

Laura C. Rodrigues, MD, PhD^d

Peter G. Kremsner, MD^{e,f}

Maria Yazdanbakhsh, PhD^b

Dieter Kube, PhD^{c,f}

From ^athe Telethon Institute for Child Health Research, Center for Child Health Research, University of Western Australia, Perth; ^bthe Department of Parasitology, Leiden University Medical Center, The Netherlands; ^cthe Hematology/Oncology Division, Georg-August-University, Göttingen, Germany; ^dthe Department of Epidemiology and Population Sciences, School of Hygiene and Tropical Medicine, London, United Kingdom; ^ethe Medical Research Unit, Albert Schweitzer Hospital, Lambarene, Gabon; and ^fthe Department of Parasitology, Institute for Tropical Medicine, Eberhard-Karls-University, Tübingen, Germany. E-mail: anitav@ichr.uwa.edu.au.

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TABLE E1. Distribution of genotypes and alleles of 9 polymorphisms in the 5' flanking region of the human IL-10 gene in 75 HDM-SPT negative and 25 positive Gabonese schoolchildren*

Genotypes						Alleles					
	Total (n = 100)	SPT ^{neg} (n = 75)	SPT ^{pos} (n = 25)	OR for HDM-SPT (95% CI)	P value		Total (n = 200)	SPT ^{neg} (n = 150)	SPT ^{pos} (n = 50)	OR for HDM-SPT (95% CI)	P value
−7400											
in/in	76 (0.76)	59 (0.79)	17 (0.68)	1 (reference)	.483	in	174 (0.87)	133 (0.89)	41 (0.82)	1 (reference)	.450
in/del	22 (0.22)	15 (0.20)	7 (0.28)	1.48 (0.50-4.37)	.640	del	26 (0.13)	17 (0.11)	9 (0.18)	1.42 (0.57-3.55)	
del/del	2 (0.02)	1 (0.01)	1 (0.04)	2.01 (0.11-37.07)							
−6752											
AA	48 (0.48)	38 (0.51)	10 (0.40)	1 (reference)		A	140 (0.70)	110 (0.73)	30 (0.60)	1 (reference)	.103
AT	44 (0.44)	34 (0.45)	10 (0.40)	1.04 (0.38-2.86)	.938	T	60 (0.30)	40 (0.27)	20 (0.40)	1.77 (0.89-3.53)	
TT	8 (0.08)	3 (0.04)	5 (0.20)	6.02 (1.17-31.05)	.032						
−6208											
GG	36 (0.36)	29 (0.39)	7 (0.28)	1 (reference)		G	122 (0.61)	97 (0.65)	25 (0.50)	1 (reference)	.055
GC	50 (0.50)	39 (0.52)	11 (0.44)	4.46 (1.13-17.63)	.033	C	78 (0.39)	53 (0.35)	25 (0.50)	1.92 (0.99-3.72)	
CC	14 (0.14)	7 (0.09)	7 (0.28)	1.23 (0.42-3.61)	.712						
−3538											
TT	50 (0.50)	40 (0.53)	10 (0.40)	1 (reference)		T	143 (0.71)	113 (0.75)	30 (0.60)	1 (reference)	.051
TA	43 (0.43)	33 (0.44)	10 (0.40)	10.28 (1.60-65.99)	.014	A	57 (0.29)	37 (0.25)	20 (0.40)	2.00 (1.00-4.01)	
AA	7 (0.07)	2 (0.03)	5 (0.20)	1.15 (0.42-3.16)	.784						
−2736											
CC	24 (0.24)	20 (0.27)	4 (0.16)	1 (reference)		C	109 (0.55)	88 (0.59)	21 (0.42)	1 (reference)	.037
CA	59 (0.60)	46 (0.61)	13 (0.52)	5.86 (1.29-26.59)	.022	A	91 (0.45)	62 (0.41)	29 (0.58)	2.03 (1.04-3.95)	
AA	16 (0.16)	8 (0.11)	8 (0.32)	1.52 (0.43-5.34)	.517						
−1354											
GG	36 (0.36)	30 (0.40)	6 (0.24)	1 (reference)		G	123 (0.61)	99 (0.66)	24 (0.48)	1 (reference)	.045
GA	51 (0.51)	39 (0.52)	12 (0.48)	5.60 (1.34-23.41)	.018	A	77 (0.39)	51 (0.34)	26 (0.52)	1.97 (1.01-3.82)	
AA	13 (0.13)	6 (0.08)	7 (0.28)	1.41 (0.47-4.29)	.541						
−1087											
AA	34 (0.34)	27 (0.36)	7 (0.28)	1 (reference)		A	120 (0.60)	96 (0.64)	24 (0.48)	1 (reference)	.060
AG	52 (0.52)	42 (0.56)	10 (0.40)	0.86 (0.29-2.60)	.791	G	80 (0.40)	54 (0.36)	26 (0.52)	1.88 (0.97-3.65)	
GG	14 (0.14)	6 (0.08)	8 (0.32)	5.64 (1.39-22.95)	.016						
−824											
CC	33 (0.33)	20 (0.27)	13 (0.52)	1 (reference)		C	123 (0.61)	87 (0.58)	36 (0.72)	1 (reference)	.097
CT	57 (0.57)	47 (0.63)	10 (0.40)	0.34 (0.13-0.94)	.037	T	77 (0.39)	63 (0.42)	14 (0.28)	0.55 (0.28-1.06)	
TT	10 (0.10)	8 (0.11)	2 (0.08)	0.37 (0.07-2.11)	.265						
−597											
CC	33 (0.33)	20 (0.27)	13 (0.52)	1 (reference)		C	123 (0.61)	87 (0.58)	36 (0.72)	1 (reference)	.097
CA	57 (0.57)	47 (0.63)	10 (0.40)	0.34 (0.13-0.94)	.037	A	77 (0.39)	63 (0.42)	14 (0.28)	0.55 (0.28-1.06)	
AA	10 (0.10)	8 (0.11)	2 (0.08)	0.37 (0.07-2.11)	.265						

*Associations between genotypes or alleles of individual polymorphisms and SPT reactivity were determined by using logistic regression models adjusting for age and sex.

TABLE E2. Association between alleles of individual polymorphisms in the 5' flanking region of the human IL-10 gene and IL-10 production in 100 Gabonese schoolchildren*

	IL-10-PHA					
	Total population (n = 100)		SPT ^{neg} (n = 75)		SPT ^{pos} (n = 25)	
	Median (IQR)	P value	Median (IQR)	P value	Median (IQR)	P value
–7400						
in	330 (203-443)	.274	331 (232-452)	.451	257 (146-363)	.706
del	307 (103-482)		312 (3-488)		169 (121-425)	
–6752						
A	330 (207-450)		338 (232-477)		268 (154-353)	
T	307 (159-449)	.303	321 (203-470)	.429	203 (114-419)	.692
–6208						
G	330 (229-452)		338 (238-478)		279 (202-356)	
C	307 (156-441)	.200	330 (194-464)	.339	169 (129-402)	.534
–3538						
T	329 (203-443)		331 (232-452)		268 (154-353)	
A	324 (151-478)	.514	330 (191-482)	.831	203 (113-419)	.692
–2736						
C	338 (232-476)		356 (239-479)		279 (203-363)	
A	285 (137-429)	.014	296 (184-438)	.062	169 (107-370)	.125
–1354						
G	331 (232-442)		349 (237-452)		301 (202-361)	
A	285 (158-478)	.157	302 (203-479)	.349	185 (118-396)	.331
–1087						
A	331 (238-450)		353 (239-479)		279 (167-363)	
G	288 (157-446)	.053	307 (184-458)	.095	223 (133-370)	.586
–824						
C	290 (169-443)		312 (203-452)		245 (126-365)	
T	338 (239-466)	.062	356 (239-484)	.175	303 (190-380)	.243
–597						
C	290 (169-443)		312 (203-452)		245 (126-365)	
A	338 (239-466)	.062	356 (239-484)	.175	303 (190-380)	.243

IQR, Interquartile range.

*Differences between *in vitro* PHA-induced IL-10 levels for 9 polymorphic IL-10 alleles were tested for the total population and for HDM-SPT negative (n = 75) and positive (n = 25) children separately by using the nonparametric Mann-Whitney test.