HHIP, *HDAC4*, *NCR3* and *RARB* polymorphisms affect fetal, childhood and adult lung function

To the Editor:

Impaired lung function, and consequent respiratory morbidity including asthma and chronic obstructive pulmonary disease, may have their origins in early life [1–3]. Genome-wide analysis studies (GWAS) have identified a number of single-nucleotide polymorphisms (SNPs) in those of European ancestry that affect adult lung function, as measured by forced expiratory volume in 1 s (FEV_1) and FEV_1/forced vital capacity (FVC) ratio. 23 of these SNPs have directionally consistent effects on both FEV_1 and FEV_1/FVC in children and adults [4].

TABLE 1 Single-nucleotide polymorphism (SNP) code, target gene, chromosome and minor allele of the 23 analysed SNPs associated with adult lung function and previously showing directionally consistent effects in children, and SNPs and their target genes showing significant associations with lung function in infants along with the mean values of the relevant parameter according to the minor allele count

SNP	Gene	Chromosome	Minor allele	AA	Aa	aa
rs993925	*TGFB2*	1	T	55	57	22
rs12477314	*HDAC4*	2	T	87	39	5
rs1529672	*RARB*	3	C	93	38	0
rs1344555	*MECOM*	3	T	80	45	7
rs153916	*SPATA9*	5	T	46	66	18
rs6903823	*ZKSCAN3/ZNF323*	6	G	63	57	12
rs2857595	*NCR3*	6	G	76	52	0
rs2798641	*ARMC2*	6	T	95	37	3
rs7068966	*CDC123*	10	T	85	42	2
rs11001819	*C10orf11*	10	G	41	56	0
rs11172113	*LRP1*	12	T	51	64	18
rs1036429	*CCDC38*	12	T	38	66	0
rs12447804	*MMP15*	16	T	76	55	4
rs2571445	*TNS1*	2	G	51	62	23
rs10498230	*PID1*	2	T	105	26	2
rs2045517	*FAM13A*	4	T	45	65	26
rs10516526	*GSTCD*	4	G	117	18	0
rs17331332	*NPNT*	4	G	116	18	0
rs6823809	*NPNT*	4	T	66	59	11
rs1032296	*HHIP*	4	T	38	73	21
rs11100860	*HHIP*	4	G	50	68	18
rs11134779	*ADAM19*	5	G	53	64	16
rs2070600	*AGER*	6	T	113	20	0

SNP	Gene	Minor allele	Effect on infant lung function	Minor allele count	Mean	β	p-value
rs12477314 (downstream)	*HDAC4*	T	↑ compliance	0	47.4	0.07	0.02
				1	51.0		
				2	58.1		
rs12477314 (downstream)	*HDAC4*	T	↑ $V'_{max,FRC}$	0	136.3	0.18	0.02
				1	146.4		
				2	233.1		
rs2857595 (upstream)	*NCR3*	G	↓ respiratory rate	0	46.3	-0.06	0.04
				1	43.0		
				2	NA		
rs1529672 (intron)	*RARB*	C	↑ $V'_{max,FRC}$	0	133.1	0.20	0.03
				1	161.1		
				2	NA		
rs11100860 (upstream)	*HHIP*	T	↑ compliance	0	45.2	0.08	<0.001
				1	50.3		
				2	51.9		
rs1032296 (upstream)	*HHIP*	G	↓ compliance	0	51.8	-0.06	0.02
				1	47.5		
				2	46.7		

Frequency of major allele (A) and minor allele (a) homozygotes/heterozygotes in our study is also shown. Effect size is shown as the β-coefficient following logarithmic transformation and regression, along with the associated p-value. *TGFB2*: transforming growth factor β2; *HDAC4*: histone deacetylase 4; *RARB*: retinoic acid receptor β; *MECOM*: MDS1 and EVI1 complex; *SPATA9*: spermatogenesis-associated protein 9; *ZKSCAN3*: zinc finger with KRAB and SCAN domains 3; *ZNF323*: zinc finger protein 323; *NCR3*: natural cytotoxicity triggering receptor 3; *ARMC2*: armadillo repeat-containing protein 2; *CDC123*: cell division cycle homologue 123; *C10orf11*: chromosome 10 open reading frame 11; *LRP1*: low-density lipoprotein receptor-related protein 1; *CCDC38*: coiled coil domain-containing protein 38; *MMP15*: matrix metalloprotease 15; *TNS1*: tensin 1; *PID1*: phosphotyrosine interaction domain-containing protein 1; *FAM13A*: family with sequence similarity 13, member A; *GSTCD*: glutathione *S*-transferase, C-terminal domain-containing protein; *NPNT*: nephronectin; *HHIP*: hedgehog interacting protein; *ADAM19*: a disintegrin and metalloprotease 19; *AGER*: advance glycosylation and end produce-specific receptor; $V'_{max,FRC}$: maximal expiratory flow at functional residual capacity: NA: not applicable (no subjects with two minor alleles).

During 1998–2002, the Southampton Women's Survey recruited 12,579 females pre-conception through their general practitioners [5]. By the end of 2003, there had been 1,973 babies born to these females, of which 147 had infant lung function measured between 5 and 14 weeks of age, according to previously published protocols [6] using raised volume/rapid compression techniques to measure maximal expiratory flow at functional residual capacity ($V'_{max,FRC}$), $FEV_{0.4}$, respiratory rate and compliance. DNA was obtained from cord blood samples or from buccal samples taken at the 6-yr follow-up. DNA from these 147 children was analysed for each of the 23 SNPs identified as above. These SNPs are detailed in table 1.

Linear regression was used to analyse the minor allele count for each SNP (either 0, 1 or 2) against logarithmically transformed and age-adjusted values for infant lung compliance, respiratory rate, $FEV_{0.4}$ and $V'_{max,FRC}$. Smoking in pregnancy, maternal body mass index, social class, birth weight, gestation and crown–rump length were analysed as potential confounding factors. The average n per group (0, 1 or 2 minor alleles) across all 23 SNPs was 71, 50 and 9, respectively, giving 80% power to detect a 3.7-$mL \cdot mmH_2O^{-1}$ change in compliance per increase in minor allele count.

Five SNPs, relating to four genes, showed significant associations with infant lung function (table 1). Hedgehog interacting protein (*HHIP*) had one SNP (rs11100860) that was associated with increased compliance ($p<0.001$) and one (rs1032296) associated with decreased compliance ($p<0.05$). Retinoic acid receptor β (*RARB*) (rs1529672) was associated with increased $V'_{max,FRC}$ ($p<0.05$), the natural cytotoxicity triggering receptor 3 (*NCR3*) SNP (rs2857595) was associated with a lower respiratory rate ($p<0.05$), and the histone deacetylase 4 (*HDAC4*) SNP (rs12477314) was associated with both increased compliance and $V'_{max,FRC}$ (both $p<0.05$). Table 1 summarises these findings. These effects were all directionally consistent with the previous GWAS.

HHIP is known to have a role in lung development through fibroblast growth factor 10 (*FGF10*) and its control of lung branching [7], while RARB regulates lung bud formation and branching through the Wnt pathway [8] with retinoic acid playing a central role in pre- and post-natal lung development in humans [9]. HDAC4 and NCR3 have uncertain roles in lung development, though the former may modulate epigenetic effects on lung function.

Branching of the lung occurs in the pseudoglandular phase and is complete by 16 weeks of gestation [10]; therefore, RARB and HHIP are likely to have their effects in the first trimester. Early branching is the primary determinant of resistance in normal lungs and, therefore, of compliance. This large airway function is reflected as FEV_1 and FEV_1/FVC in later life; thus, there is a scientifically plausible link between these SNPs and lung function.

As there was *a priori* evidence of association between these SNPs and lung function, we have not corrected for the number of SNPs and lung function tests. However, even if a Bonferroni correction is applied (23 SNPs × 4 lung function measurements), the rs11100860 *HHIP* SNP remains significant. As the original GWAS was in similar populations to our cohort, we feel it reasonable to assume the key SNPs identified may be good proxy markers of the causal locus. It is also possible that the present study was underpowered to detect significant associations between infant lung function and the other SNPs tested.

We accept that small numbers and multiple testing are limitations of our study; however, these results may link early fetal lung development, through infant lung function, to adult lung function and respiratory morbidity in later life. This is an interesting starting point for identification of the mechanisms of fetal origins of lung function.

Samuel A. Collins[*,#], Jane S.A. Lucas[*,#], Hazel M. Inskip[¶,+], Keith M. Godfrey[¶,+,§], Graham Roberts[*,#,¶], John W. Holloway[*,¶] and the Southampton Women's Survey Study Group[+]

*Clinical and Experimental Sciences Academic Unit, University of Southampton Faculty of Medicine, [¶]Human Development and Health Academic Unit, University of Southampton Faculty of Medicine, [#]NIHR Southampton Respiratory Biomedical Research Unit, University of Southampton and University Hospital Southampton NHS Foundation Trust, [§]NIHR Southampton Biomedical Research Centre, University of Southampton and University Hospital Southampton NHS Foundation Trust, and [+]Southampton Medical Research Council Lifecourse Epidemiology Unit, University of Southampton, Southampton, UK.

Correspondence: S.A. Collins, Human Genetics and Medical Genomics, University of Southampton Faculty of Medicine, Duthie Building, MP808, Southampton General Hospital, Southampton, SO16 6YD, UK. E-mail: samacollins@gmail.com

Statement of Interest: A statement of interest for K.M. Godfrey can be found at www.erj.ersjournals.com/site/misc/statements.xhtml

REFERENCES

1 Håland G, Carlsen KCL, Sandvik L, *et al.* Reduced lung function at birth and the risk of asthma at 10 years of age. *N Engl J Med* 2006; 355: 1682–1869.
2 Stern DA, Morgan WJ, Wright AL, *et al.* Poor airway function in early infancy and lung function by age 22 years: a non-selective longitudinal cohort study. *Lancet* 2007; 370: 758–764.
3 Krauss-Etschmann S, Bush A, Bellusci S, *et al.* Of flies, mice and men: a systematic approach to understanding the early life origins of chronic lung disease. *Thorax* 2012 [in press DOI: 10.1136/thoraxjnl-2012-201902].
4 Artigas MS, Loth DW, Wain LV, *et al.* Genome-wide association and large-scale follow up identifies 16 new loci influencing lung function. *Nat Genet* 2011; 43: 1082–1090.
5 Inskip HM, Godfrey KM, Robinson SM, *et al.* Cohort profile: the Southampton Women's Survey. *Int J Epidemiol* 2006; 35: 42–48.
6 Lucas JS, Inskip HM, Godfrey KM, *et al.* Small size at birth and greater postnatal weight gain: relationships to diminished infant lung function. *Am J Respir Crit Care Med* 2004; 170: 534–540.
7 Warburton D, Bellusci S, Del Moral PM, *et al.* Growth factor signaling in lung morphogenetic centers: automaticity, stereotypy and symmetry. *Respir Res* 2003; 4: 5.
8 Kam RKT, Deng Y, Chen Y, *et al.* Retinoic acid synthesis and functions in early embryonic development. *Cell Biosci* 2012; 2: 11.
9 Biesalski HK, Nohr D. Importance of vitamin-A for lung function and development. *Mol Aspects Med* 2003; 24: 431–440.
10 Kitaoka H, Burri PH, Weibel ER. Development of the human fetal airway tree: analysis of the numerical density of airway endtips. *Anat Rec* 1996; 244: 207–213.

DOI: 10.1183/09031936.00171712