Published in final edited form as:
Eur Respir J. 2013 March ; 41(3): 756–757. doi:10.1183/09031936.00171712.

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

Samuel A Collins, MRCPCH[1,2], **Jane SA Lucas, FRCPCH PhD**[1,2], **Hazel M Inskip, PhD**[3,4], **Keith M Godfrey, FRCP PhD**[3,4,5], **Graham Roberts, MRCPCH, DM**[1,2,3], **John W Holloway, PhD**[1,3], and **the Southampton Women's Survey Study Group**[4]

[1]Clinical and Experimental Sciences Academic Unit, University of Southampton Faculty of Medicine, Southampton, UK

[2]NIHR Southampton Respiratory Biomedical Research Unit University of Southampton and University Hospital Southampton NHS Foundation Trust, Southampton, UK

[3]Human Development and Health Academic Unit, University of Southampton Faculty of Medicine, Southampton, UK

[4]Southampton Medical Research Council Lifecourse Epidemiology Unit, University of Southampton, Southampton, UK

[5]NIHR Southampton Biomedical Research Centre, University of Southampton and University Hospital Southampton NHS Foundation Trust, Southampton, UK

To the Editor;

Impaired lung function, and consequent respiratory morbidity including asthma and chronic obstructive pulmonary disease (COPD), 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 FEV_1 and FEV_1/FVC ratio. 23 of these SNPs have directionally consistent effects on both FEV1 and FEV_1/FVC in children and adults[4].

During 1998-2002, the Southampton Women's Survey (SWS) recruited 12,579 women pre-conception through their general practitioners[5]. By the end of 2003 there had been 1973 babies born to these women, 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 V'maxFRC, $FEV_{0.4}$, respiratory rate and compliance. DNA was obtained from cord blood samples or from buccal samples taken at the 6 year follow-up. DNA from these 147 children were analysed for each of the 23 SNPs identified as above. These SNPs are detailed in supplementary 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'maxFRC. Smoking in pregnancy, maternal BMI, 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 3.7ml/mmH_2O change in compliance per increase in minor allele count.

Corresponding Author Dr Samuel Collins c/o John W Holloway Human Genetics and Medical Genomics University of Southampton Faculty of Medicine Duthie Building, MP808, Southampton General Hospital Southampton, SO16 6YD Tel: +44 (0)7815 494683 Fax +44 (0)23 8079 4264 samacollins@gmail.com.

Europe PMC Funders Author Manuscripts

Five SNPs, relating to four genes, showed significant associations with infant lung function (Table I). 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 (RARβ) (rs1529672) was associated with increased V'maxFRC ($p<0.05$), 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'maxFRC (both $p<0.05$). Table 1 summarises these findings. These effects were all directionally consistent with the previous GWAS analysis.

HHIP is known to have a role in lung development through fibroblast growth factor 10 (FGF10) and its control of lung branching[7], whilst RARβ regulates lung bud formation and branching through the Wnt pathway[8] with retinoic acid playing a central role in pre- and postnatal 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 RARβ 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 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 had been applied (23 SNPs x 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 we are underpowered to detect significant associations between infant lung function and the other SNPs tested.

We accept that small numbers and multiple testing are limitations; 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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; 355:1682–9. [PubMed: 17050892]
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; 370:758–64. [PubMed: 17765525]
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. 2012:1–6. [PubMed: 22170222]
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; 43:1082–90. [PubMed: 21946350]
5. Inskip HM, Godfrey KM, Robinson SM, et al. Cohort profile: The Southampton Women's Survey. Int J Epidemiol. 2006; 35:42–8. [PubMed: 16195252]

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; 170:534–40. [PubMed: 15172897]
7. Warburton D, Bellusci S, Del Moral P-M, et al. Growth factor signaling in lung morphogenetic centers: automaticity, stereotypy and symmetry. Respir Res. 2003; 4:5. [PubMed: 12818006]
8. Kam RKT, Deng Y, Chen Y, et al. Retinoic acid synthesis and functions in early embryonic development. Cell Biosci. 2012; 2:11. [PubMed: 22439772]
9. Biesalski HK, Nohr D. Importance of vitamin-A for lung function and development. Mol Aspects Med. 2003; 24:431–40. [PubMed: 14585314]
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; 244:207–13. [PubMed: 8808395]

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Table 1

Single nucleotide polymorphisms (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, n/a indicates there were no subjects with 2 minor alleles. Effect size is shown as beta co-efficient following log transformation and regression, along with associated p value. Histone deacetylase 4 (HDAC4), natural cytotoxic receptor 3 (NCR3), retinoic acid receptor beta (RARβ), hedgehog interacting protein (HHIP).

SNP	Gene	Minor Allele	Effect on Infant Lung Function	Minor allele count	Mean	Beta	p value
rs12477314 (downstream)	HDAC4	T	↑Compliance	0 1 2	47.4 51.0 58.1	0.07	0.02
rs12477314 (downstream)	HDAC4	T	↑ V'maxFRC	0 1 2	136.3 146.4 233.1	0.18	0.02
rs2857595 (upstream)	NCR3	G	↓RR	0 1 2	46.3 43.0 n/a	−0.06	0.04
rs1529672 (intron)	RARβ	C	↑ V'maxFRC	0 1 2	133.1 161.1 n/a	0.20	0.03
rs11100860 (upstream)	HHIP	T	↑Compliance	0 1 2	45.2 50.3 51.9	0.08	<0.001
rs1032296 (upstream)	HHIP	G	↓ Compliance	0 1 2	51.8 47.5 46.7	−0.06	0.02