

Original article

Contribution of functional variation in the *IL13* gene to allergy, hay fever and asthma in the NSHD longitudinal 1946 birth cohort

Background: Genetic variants of the two adjacent genes, *IL13* and *IL4* have frequently been reported as being associated with susceptibility to atopy and asthma, both in adults and children, and some studies also suggest association with lung function and chronic obstructive pulmonary disease.

Methods: In this study, we examined for the first time the effect of these variants in 2918 adults in a longitudinal birth cohort, the British National Survey of Health and Development, where there are extensive life style, developmental and environmental data. We examine two *IL13* single nucleotide polymorphisms (SNPs) *IL13* rs20541 (R110Q) and rs1800925 (−1024C > T) and one *IL4* SNP, rs2070874 (−33C > T) with likely function.

Results: We show that *IL13* rs20541 and rs1800925 are each significantly associated with self-reported asthma and allergy, and that this association is not confounded by any of the known developmental and environmental risk factors for asthma and atopy, including in particular place of birth. *IL13* rs20541 does however act as a confounder for the *IL13* rs1800925 associations, meaning that there is no statistical support for rs1800925 having an independent effect. There is nevertheless evidence for interaction between smoking and rs1800925, with allergy as outcome. None of the SNPs showed association with measures of lung function, nor any interaction with the effect of smoking on lung function.

Conclusion: In a longitudinal population cohort we have established a role for polymorphism of *IL13* in determining susceptibility to both atopy and asthma.

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Asthma results from the effects of environmental stimuli in genetically-susceptible individuals (1), with a suggested overall genetic contribution of around 50–60% (2). Variations in many different genes have been reported to confer increased susceptibility, with two of the most frequently replicated being those encoding the interleukins (IL)-4 and IL-13 (3, 4). Interleukin 4, and also IL-13, are immunoregulatory cytokines produced by activated T helper 2 (Th2) cells and both can switch B cells to produce immunoglobulin E (IgE). The majority of asthma is associated with atopy, and is characterized by enhanced T-cell secretion of Th2 cytokines in the airways. In addition, chronic asthma is characterized by epithelial

damage, increased mucus production and airway remodelling (5), IL-4 and IL-13 being key regulators of this response, with wide ranging effects on epithelial cells, fibroblasts and smooth muscle. Evidence for a critical role for IL-13 in asthma also comes from experimental models of allergic asthma (6–8).

A number of single nucleotide polymorphisms (SNPs) have been described in the *IL13* and *IL4* genes, which have been shown to be associated with increased total serum IgE levels, as well as the development of atopy and asthma, in many but not all studies. Several, but not all of the SNPs previously studied are probably functionally significant. For example, the amino-acid substitution

IL13 R110Q (rs20541) polymorphism for which there is the best evidence of association with increase in IgE (9–12) and asthma (13–16), has been suggested by molecular modelling (14) and experimentally (17) to be critical to ligand-binding. The *IL13* 110Q allele has also been shown to induce significantly greater IgE switching than *IL13* 110R in a cellular model (18). This SNP was therefore selected as our first choice for analysis. We also elected to study two other candidates rs1800925 (*IL13* 5' –1024C > T) for which there is evidence of regulatory function (19), and rs2070874 in the adjacent gene, *IL4* (*IL4* 5' –33C > T), postulated to be in a *cAMP response element binding transcription factor* (CREB)-binding site (20). As all three SNPs are located within a 20 kb region, linkage disequilibrium (LD) was measured and haplotypes constructed.

Most information for the role of these SNPs in asthma susceptibility so far obtained comes from case-control studies or families, although association of *IL13* and *IL4* with IgE levels was reported for the British 1958 longitudinal birth cohort (12) and a recent study examined the association between *IL13* and childhood asthma in the 1989 Isle of Wight birth cohort (21). Using data from the nationally representative 1946 British birth cohort study, we investigated the role of variation at the *IL13/IL4* loci in conferring genetic risk of asthma, allergy and hay fever.

As some studies report association with chronic obstructive pulmonary disease (19) and with lung function in smokers (22), we also considered spirometry measures as additional outcome variables. Extensive lifestyle, developmental and environmental data, as well as health information derived from questionnaires, has been collected prospectively, so that known nongenetic risk factors can be considered as potential confounders.

We also investigated the possibility of gene-environment interactions. Smoking was of particular interest as IL-13 levels have been shown to be increased in children whose parents smoke (23) and an interaction between *IL13* rs1800925 and smoking with childhood asthma as the outcome has been reported (21). In addition, there is evidence for this same SNP modulating the adverse affect of smoking on lung function in adults (22). It is known that lower respiratory tract infections in childhood predispose to asthma, and it is possible that this risk is influenced by *IL13* genotype, as production of IL-13 during viral infections may alter the amount of virus-induced inflammation (24–26). Confounding and interaction of the IL-13 SNPs with each other were also tested.

Methods

Study

The MRC National Survey of Health and Development (NSHD) is a longitudinal birth cohort study consisting of a socially stratified

sample of all births in England, Scotland and Wales in 1 week in March 1946. The original sample consisted of 2547 women and 2815 men and there was very little ethnic variation in this cohort, as 1946 was before the 20th century mass immigration into Britain. This sample was studied at birth and then a further 10 times up to age 15 years, and then 11 more times in adulthood, most recently at 53 years when 3035 cohort members (1472 men, 1563 women) provided information. The majority ($n = 2989$) were interviewed and examined in their own homes by research nurses, with others completing a postal questionnaire ($n = 46$). Contact was not attempted for the 1979 individuals who had previously refused to take part, were living abroad, were untraced since last contact at 43 years or had already died. The responding sample at the age of 53 is in most respects representative of the national British-born population of a similar age (27). The data collection at age 53 received ethics committee approval (MREC no 98/2/121), and informed consent was obtained for each set of questions and measures.

DNA extraction

Collection of buccal samples and extraction of DNA ($n = 2939$), as well as validation of the samples has been described previously (28).

IL-13/IL-4 SNP typing

The three SNPs tested were *IL13* rs20541, *IL13* rs1800925 and *IL4* rs2070874. Details of the genotyping methods are provided in the Supporting Information. In each case, genotype was inferred from observed phenotype (viz. A, GA and G were interpreted as AA GA and GG). Typings were validated by blind retesting and typing control samples previously genotyped by other methods. The 'call rate' for rs20541 was over 99%, while those of rs2070874 and rs1800925 which used a multiplex method were 95%.

Genetic analysis

The program LDMax, which uses the Expectation-Maximization (EM) algorithm to infer phase was used to measure LD (D') (29). Haplotype frequencies, estimated using EM, were obtained from the software package ARLEQUIN (30). Individual pairwise IL-13 haplotypes were inferred by the Bayesian method, PHASE (31).

Outcome variables

The outcome variables were derived from responses to questions about asthma, hay fever and allergy asked by the research nurses at home visits in 1989 and 1999. For each of these conditions, in 1989, cohort members were asked whether they had ever had the condition, and in 1999 they were asked whether they had had it in the last 10 years. Any cohort member reporting having the condition at either visit was considered as a case in these analyses. Forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) were measured at these same home visits, using the Micromedical turbine electronic spirometer (Cardinal Health UK 232 Ltd, Basingstoke, UK). Two measures were recorded at each visit and the maximum readings were used in this study. The following measures were then calculated: the ratio (FEV_1/FVC); the decline in FEV_1 from age 43 to 53 defined as the difference between the maximum FEV_1 values at ages 43 and 53.

Potential confounders

Birth weight, early growth, lower respiratory tract infection in childhood, breast feeding, atmospheric pollution from coal burning in childhood, childhood social class and own adult social class, body mass index (BMI) and obesity, parental and own smoking habits, region of birth and gender were identified from the literature as potential confounding variables (23, 32–34). Birth weight was extracted by mid-wives and health visitors from records within a few weeks of delivery and converted into kilograms. Weight and height were measured at ages 2 and 7 years. Lower respiratory tract infection, reported by mothers at interviews with health visitors during childhood, was coded as ever/never had. Mothers reported breast feeding history when the cohort members were aged 2 years and this was coded: never; stopped after 4 months; breast fed for more than 5 months. Atmospheric pollution combined levels of pollution from coal burning in 1946 and 1948. Childhood social class was based on the occupation of the father in 1950 when the cohort member was aged 4 years. Information on cohort members own current or most recent occupation was obtained from reports to the research nurses at the home interviews at age 53 years, from which the Registrar General's social class categories were derived. Both social class variables were coded as nonmanual/manual. Parental and own smoking habits were from reports by cohort members at the home interviews at age 53 years. Parental smoking was coded according to whether none, one or both parents had smoked and own smoking was categorized as ever/never smoked. At these visits, the nurses also measured height (using a portable CMS stadiometer, Chasmore Ltd, London, UK) and weight, from which BMI was calculated (and obesity defined as ≥ 30 kg/m²). Region of birth, originally categorized into 11 regions was collapsed into four categories: Scotland; northern regions; Wales, Midlands, Eastern, Southern regions; London and South East, and confounding was tested using these categories and for rs20541 also the original 11 regions.

Statistical methods for epidemiological analysis

The association between each genetic variable and each binary outcome (asthma, hay fever, allergy) was assessed using chi-squared tests. The SNPs were analysed as three genotypes and the *IL13* risk haplotype carrier status as a binary variable. Logistic regression models were then used to assess the impact of the potential confounding variables on the *IL13* genetic variables. For each of the three outcomes, each potential confounder was added separately to the model including only the genetic variable and the extent of change in the association between genotype status and outcome was assessed.

Tests for interaction between smoking status and *IL13* genotype status were carried out to assess whether the association between *IL13* and each of the outcomes was significantly different among 'ever' smokers compared with 'never' smokers. Tests for interaction between the presence, or not, of lower respiratory tract infections in childhood and *IL13* were conducted to determine whether the association between *IL13* and asthma is significantly different according to respiratory tract infection status.

We then tested the association between each genetic variable and the outcomes FEV₁/FVC, FEV₁, and decline in FEV₁. Multiple normal regression models adjusted for gender and height were used in the analyses for these outcomes. For each of these outcomes, tests of interaction between smoking status and the genetic variables were carried out.

Analyses were carried out in SPSS version 14 (SPSS Inc., Chicago, IL, USA) and STATA version 10 (StataCorp LP., College Station, TX, USA). A *P*-value of ≤ 0.05 was considered to be statistically significant.

Table 1. Allele and genotype frequencies in the samples analysed and *P*-value for deviation from Hardy–Weinberg equilibrium

Marker	Location and type	N	MAF	N for each genotype			P-value
<i>IL13</i> rs20541	Exon 4	2905	0.17	GG 2002	GA 815	AA 88	0.90
<i>IL13</i> rs1800925	Promoter	2795	0.18	CC 1865	CT 834	TT 96	0.97
<i>IL4</i> rs2070874	Promoter	2788	0.12	CC 2164	CT 590	TT 34	0.68

MAF, minor allele.

Results

Genotype and allele frequency data

Genotype and allele frequency results are shown in Table 1. There was no significant deviation from Hardy–Weinberg equilibrium for any of the three SNPs, and the genotype frequencies were found to be very similar in people born in different parts of the UK (data not shown).

Table 2A shows pairwise *D'* values for each of the markers. All three SNPs are significantly associated with each other, with a high LD between *IL13* rs20541 and *IL13* rs1800925. PHASE was used to infer haplotypes for the two *IL13* SNPs and the results are shown in Table 2B. The EM algorithm returned similar (but not identical) haplotype frequencies.

Associations with disease outcomes

Data were available for at least one of the SNPs and one or more of the disease outcomes for 2742 of those who donated DNA. Table 3 shows the results of initial cross-tabulation analyses of *IL13* rs20541, *IL13* rs1800925, *IL4* rs2070874 and asthma, allergy and hay fever. For both

Table 2. (A) Pairwise linkage disequilibrium (LD, measure *D'*) between the SNPs, *IL13* rs20541, *IL13* rs1800925 and *IL4* rs2070874. (B) Haplotypes for markers rs20541/rs1800925

(A)				
SNP pair	N	χ^2	P-value	<i>D'</i>
rs20541/rs1800925	2794	706.54	0.00000	0.520
rs20541/rs2070874	2795	18.23	0.00002	0.037
rs1800925/rs2070874	2787	93.19	0.00000	0.240

Values derived using LDMax, which uses the EM algorithm to infer phase.

(B)			
Haplotype rs20541/rs1800925	Number PHASE	Frequency PHASE	Frequency EM
GT	409	0.073	0.079
AT	616	0.110	0.105
GC	4212	0.754	0.749
AC	349	0.062	0.068

Number and frequency of haplotypes inferred by PHASE, numbers (confidence of more than 0.94) and frequencies derived using the EM algorithm.

Table 3. Unadjusted associations between outcomes and genotypes for different genetic risk factors

(A) *IL13* rs20541

Outcome	Yes/total (%)	<i>IL13</i> rs20541 genotypes <i>n/N</i> (%)			<i>P</i> -value homogeneity	<i>P</i> -value trend
		GG	GA	AA		
Ever asthma	275/2728 (10.1)	166/1895 (8.8)	98/755 (13.0)	11/78 (14.1)	0.002	0.001
Ever hay fever	651/2723 (23.9)	442/1890 (23.4)	185/755 (24.5)	24/78 (30.8)	0.3	0.2
Ever allergy	847/2714 (31.2)	557/1884 (29.6)	264/752 (35.1)	26/78 (33.3)	0.020	0.01

(B) *IL13* rs1800925

Outcome	Yes/total (%)	<i>IL13</i> rs1800925 genotypes <i>n/N</i> (%)			<i>P</i> -value homogeneity	<i>P</i> -value trend
		CC	CT	TT		
Ever asthma	263/2625 (10.0)	158/1767 (8.9)	98/771 (12.7)	7/87 (8.0)	0.01	0.04
Ever hay fever	629/2621 (24.0)	407/1764 (23.1)	199/770 (25.8)	23/87 (26.4)	0.3	0.1
Ever allergy	821/2611 (31.4)	525/1756 (29.9)	267/769 (34.7)	29/86 (33.7)	0.05	0.02

(C) *IL4* rs2070874

Outcome	Yes/total (%)	<i>IL4</i> rs2070874 genotypes <i>n/N</i> (%)			<i>P</i> -value homogeneity	<i>P</i> -value trend
		CC	CT	TT		
Ever asthma	262/2618 (10.0)	204/2036 (10.0)	56/552 (10.1)	2/30 (6.7)	0.8*	0.9
Ever hay fever	628/2614 (24.0)	487/2033 (24.0)	136/551 (24.7)	5/30 (16.7)	0.6	0.9
Ever allergy	819/2604 (31.5)	624/2026 (30.8)	191/548 (34.9)	4/30 (13.3)	0.02	0.5

*1 cell (16.7%) have expected count less than 5. The minimum expected count is 3.00.

(D) *IL13* AT haplotype carriers vs noncarriers

Outcome	Yes/total (%)	<i>IL13</i> AT haplotype carriers vs noncarriers <i>n/N</i> (%)		<i>P</i> -value homogeneity
		Yes/other	Yes/AT carriers	
Ever asthma	261/2612 (10.0)	190/2077 (9.2)	71/535 (13.3)	0.005
Ever hay fever	626/2608 (24.0)	480/2073 (23.2)	146/535 (27.3)	0.05
Ever allergy	816/2598 (31.4)	622/2065 (30.1)	194/533 (36.4)	0.005

(E) *IL-13* risk allele carriers vs noncarriers

Outcome	Yes/total (%)	<i>IL13</i> CT, AT and AC haplotype carriers vs noncarriers (GC homozygotes) <i>n/N</i> (%)		<i>P</i> -value homogeneity
		Yes/other	Yes/GC homozygotes	
Ever asthma	261/2612 (10.0)	140/1216 (12.4)	121/1486 (8.1)	<0.001
Ever hay fever	626/2608 (24.0)	277/1215 (24.6)	349/1483 (23.5)	0.52
Ever allergy	816/2598 (31.4)	386/1122 (34.4)	430/1476 (29.1)	0.004

n, number affected; *N*, totals for each genotype class.

P-values for test of homogeneity of proportions, and for test of linear trend are shown.

the *IL13* SNPs, there is statistically significant association with asthma. In each case, there is a higher frequency of the minor allele, rs20541 A and rs1800925 T in the 'yes' group as compared with the 'no' group (*IL13* rs20541: 0.21 vs 0.17 *IL13* rs1800925: 0.21 vs 0.18), which is consistent with the literature. *IL13* rs20541 was also significantly associated with self-reported allergy but not with hay fever. For *IL4* rs2070874, there was some evidence of an association with allergy, with the heterozygotes showing highest prevalence. Combination of the two *IL13* SNPs, to test carrier status for the risk AT

haplotype vs noncarrier status, also shows, as expected, a significant association with asthma and allergy. There was weak evidence of an association with hay fever ($P = 0.05$, Table 3D). Homozygosity for the *IL13* haplotype GC, which carries neither of the risk alleles, confers significantly less risk of both asthma and allergy than being a carrier for one or both (Table 3E).

There was no association between any of the *IL13/IL4* genotypes and measures of respiratory function (FEV₁, FEV₁/FVC in 1999 or decline in FEV₁ between 1989 and 1999), adjusted for gender and height (data not shown).

Tests for confounding

No evidence of confounding by nongenetic variables was observed, with all *IL13* associations with asthma, hay fever and allergy, remaining unchanged after adjustments (data not shown).

As *IL13* rs20541 is in high LD with rs1800925 (D' 0.52, Table 3) the possibility that the two genetic variables are confounded by each other was also tested. In a model including both genetic variables, only the association of *IL13* rs20541 with asthma remained statistically significant ($P = 0.02$). The OR for *IL13* rs1800925 was attenuated and the association was no longer statistically significant.

Tests for interactions

For allergy, there was some evidence of an interaction between smoking status and *IL13* rs1800925 ($P = 0.03$). Treating *IL13* rs1800925 as a continuous variable, in which a linear relationship is assumed, the association between this genetic variable increased more steeply among smokers than nonsmokers. For both asthma and hay fever, we found no evidence of interaction between either *IL13* rs20541 or *IL13* rs1800925 and smoking status and for the asthma outcome no evidence of interaction between either of these *IL13* SNPs and childhood chest disease.

There was no evidence of an interaction of smoking status with *IL13* rs20541, *IL13* rs1800925 or *IL4*, for the outcomes FEV₁/FVC, FEV₁ at 53, or 10-year decline in FEV₁ decline (data not shown).

We also tested for evidence of interactive effect of the two *IL13* SNPs (rs20541 and rs1800925) but no statistically significant interaction was found with any of the outcomes; asthma ($P = 0.15$); hay fever ($P = 0.19$) and allergy ($P = 0.99$).

Conclusions

The data presented in this study provide clear support for the role of *IL13* polymorphism on risk of asthma and allergy, and possibly hay fever, reported for the first time in an adult longitudinal cohort. The small association of the linked locus *IL4* rs2070874 with allergy was not pursued further. None of the published environmental risk factors that we tested were found to confound the associations of any of the *IL-13* genotypes with the outcomes asthma, hay fever and allergy.

The outcome measures are self-reported and we acknowledge that there may be a potential problem of misclassification of disease outcome. However, 75% of the respondents who said they had suffered from asthma had had this diagnosis confirmed by a medical practitioner and/or taken prescribed medication for it. Population stratification resulting from differences in ancestry can cause spurious associations. However, this cohort is

uniform in ethnicity, since 1946 was before the 20th century's mass immigration into Britain. Furthermore, there were no statistically significant differences in genotype frequencies according to region of birth, and region of birth did not confound any of the associations. The main strength of our study is that it is the first *IL13*/asthma study for an adult longitudinal cohort, for which there are other life style and environmental data available, which means that this and most other potential confounders can be taken into account.

There was no confounding with environmental variables, but SNP *IL13* rs20541 reduced the association of *IL13* rs1800925 with both asthma and allergy in models including both SNPs together. In such models, rs1800925 was no longer statistically significant and had no independent effect on the outcome. There was no significant association of this or the other SNPs on any of the spirometry measures, either in smokers or nonsmokers. There is little evidence of interaction, though for rs1800925, there was some evidence of an interaction with smoking for the outcome allergy. A greater increase in risk of allergy was observed for the T allele carriers among the smokers compared with the nonsmokers. As rs1800925 is located in a promoter of the gene, it is plausible that this is a real biological effect involving cigarette smoke upregulating one allele (T) more effectively than its counterpart. It should be possible for this to be tested experimentally. The effect is not seen for rs20541 suggesting that it is specific to this locus. Interestingly, in another study, the T allele of same SNP (rs1800925), has been shown to interact with the effect of smoking on FEV₁ as an outcome (22). However, quite the opposite effect was observed with maternal smoking and childhood asthma, the CC homozygotes showing greater risk of childhood asthma from maternal smoking (in pregnancy) than other genotypes (21).

In summary, our evidence suggests that the main effect of genetic variation in the *IL13* locus on asthma is via the R to Q substitution caused by the G to A transition (rs20541) (OR for asthma 1.6, CI 1.2, 2.0, for a person with one or more A alleles). Individuals homozygous for the 'nonrisk' alleles at both *IL13* loci, namely the GC haplotype homozygotes (Table 3E) appear to be most protected from asthma and allergy. There is a hint of an effect on allergy susceptibility caused by rs1800925, which is detectable in smokers only. This study illustrates the complexity of analysing genomic regions in which there is a high level of allelic association, even where there is incomplete LD. As well as overlapping effects of the genetic variants (both of which are known to affect function) the outcomes consist of partially overlapping groups of individuals. For example, the allergy outcome is likely to include people with skin allergies but no respiratory symptoms. The majority of each group is likely to have raised IgE, characteristic of atopy, although some asthmatics are not atopic. It has been shown that *IL13* rs20541 A and rs1800925 carriers have, on average

higher IgE levels, although the genetic variation accounts for a rather small proportion of the variance (12), which is also undoubtedly affected by other genes and many nongenetic factors such as smoking (35). IL-13 also acts at other levels, being associated for example with increased mucus production (36, 37), which is a symptom of asthma. There thus may be slight differences in the effect of the different alleles on these disease related changes.

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References

1. Yang IA, Savarimuthu S, Kim ST, Holloway JW, Bell SC, Fong KM. Gene-environmental interaction in asthma. *Curr Opin Allergy Clin Immunol* 2007;**7**:75–82.
2. Holloway JW, Jongepier H, Beghé B, Koppelman GH, Holgate ST, Postma DS. The genetics of asthma. *Eur Respir Monogr* 2003;**8**:26–56.
3. Ober C, Hoffjan S. Asthma genetics 2006: the long and winding road to gene discovery. *Genes Immun* 2006;**7**:95–100.
4. Vercelli D. Discovering susceptibility genes for asthma and allergy. *Nat Rev Immunol* 2008;**8**:169–182.
5. Holgate ST, Davies DE, Powell RM, Howarth PH, Haitchi HM, Holloway JW. Local genetic and environmental factors in asthma disease pathogenesis: chronicity and persistence mechanisms. *Eur Respir J* 2007;**29**:793–803.
6. Grunig G, Warnock M, Wakil AE, Venkayya R, Brombacher F, Rennick DM et al. Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 1998;**282**:2261–2263.
7. Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL et al. Interleukin-13: central mediator of allergic asthma. *Science* 1998;**282**:2258–2261.
8. Zhu Z, Homer RJ, Wang Z, Chen Q, Geba GP, Wang J et al. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J Clin Invest* 1999;**103**:779–788.
9. Graves PE, Kabesch M, Halonen M, Holberg CJ, Baldini M, Fritzsche C et al. A cluster of seven tightly linked polymorphisms in the IL-13 gene is associated with total serum IgE levels in three populations of white children. *J Allergy Clin Immunol* 2000;**105**:506–513.
10. Leung TF, Tang NL, Chan IH, Li AM, Ha G, Lam CW. A polymorphism in the coding region of interleukin-13 gene is associated with atopy but not asthma in Chinese children. *Clin Exp Allergy* 2001;**31**:1515–1521.
11. Wang M, Xing ZM, Lu C, Ma YX, Yu DL, Yan Z et al. A common IL-13 Arg130Gln single nucleotide polymorphism among Chinese atopy patients with allergic rhinitis. *Hum Genet* 2003;**113**:387–390.
12. Maier LM, Howson JM, Walker N, Spickett GP, Jones RW, Ring SM et al. Association of IL13 with total IgE: evidence against an inverse association of atopy and diabetes. *J Allergy Clin Immunol* 2006;**117**:1306–1313.
13. Chan IH, Leung TF, Tang NL, Li CY, Sung YM, Wong GW et al. Gene-gene interactions for asthma and plasma total IgE concentration in Chinese children. *J Allergy Clin Immunol* 2006;**117**:127–133.
14. Heinzmann A, Mao XQ, Akaiwa M, Kreomer RT, Gao PS, Ohshima K et al. Genetic variants of IL-13 signalling and human asthma and atopy. *Hum Mol Genet* 2000;**9**:549–559.
15. Howard TD, Whittaker PA, Zaiman AL, Koppelman GH, Xu J, Hanley MT et al. Identification and association of polymorphisms in the interleukin-13 gene with asthma and atopy in a Dutch population. *Am J Respir Cell Mol Biol* 2001;**25**:377–384.
16. Xi D, Pan S, Cui T, Wu J. Association between IL-13 gene polymorphism and asthma in Han nationality in Hubei Chinese population. *J Huazhong Univ Sci Technol Med Sci* 2004;**24**:219–222.
17. Andrews AL, Bucchieri F, Arima K, Izuhara K, Holgate ST, Davies DE et al. Effect of IL-13 receptor alpha2 levels on the biological activity of IL-13 variant R110Q. *J Allergy Clin Immunol* 2007;**120**:91–97.
18. Vladich FD, Brazille SM, Stern D, Peck ML, Ghittoni R, Vercelli D. IL-13 R130Q, a common variant associated with allergy and asthma, enhances effector mechanisms essential for human allergic inflammation. *J Clin Invest* 2005;**115**:747–754.
19. van der Pouw Kraan TC, van Veen A, Boeijs LC, van Tuyl SA, de Groot ER, Stapel SO et al. An IL-13 promoter polymorphism associated with increased risk of allergic asthma. *Genes Immun* 1999;**1**:61–65.
20. Gervaziev YV, Kaznatcheev VA, Gervazieva VB. Allelic polymorphisms in the interleukin-4 promoter regions and their association with bronchial asthma among the Russian population. *Int Arch Allergy Immunol* 2006;**141**:257–264.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Supplemental information on SNP methods. Contribution of functional variation in the *IL13* gene to allergy, hay fever and asthma in the NSHD longitudinal 1946 birth cohort.

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21. Sadeghnejad A, Karmaus W, Arshad SH, Kurukulaaratchy R, Huebner M, Ewart S. IL13 gene polymorphisms modify the effect of exposure to tobacco smoke on persistent wheeze and asthma in childhood, a longitudinal study. *Respir Res* 2008;**9**:2.
22. Sadeghnejad A, Meyers DA, Bottai M, Sterling DA, Bleecker ER, Ohar JA. IL13 promoter polymorphism 1112C/T modulates the adverse effect of tobacco smoking on lung function. *Am J Respir Crit Care Med* 2007;**176**:748–752.
23. Feleszko W, Zawadzka-Krajewska A, Matysiak K, Lewandowska D, Peradzynska J, Dinh QT et al. Parental tobacco smoking is associated with augmented IL-13 secretion in children with allergic asthma. *J Allergy Clin Immunol* 2006;**117**:97–102.
24. Copenhaver CC, Gern JE, Li Z, Shult PA, Rosenthal LA, Mikus LD et al. Cytokine response patterns, exposure to viruses, and respiratory infections in the first year of life. *Am J Respir Crit Care Med* 2004;**170**:175–180.
25. Friedlander SL, Jackson DJ, Gangnon RE, Evans MD, Li Z, Roberg KA et al. Viral infections, cytokine dysregulation and the origins of childhood asthma and allergic disease. *Pediatr Infect Dis J* 2005;**24**:S170–S176, discussion.
26. Gern JE, Brooks GD, Meyer P, Chang A, Shen K, Evans MD et al. Bidirectional interactions between viral respiratory illnesses and cytokine responses in the first year of life. *J Allergy Clin Immunol* 2006;**117**:72–78.
27. Wadsworth MEJ, Butterworth SL, Hardy RJ, Kuh DJ, Richards M, Langenberg C et al. The life course prospective design: an example of benefits and problems associated with study longevity. *Soc Sci Med* 2003;**57**:2193–2205.
28. Rousseau K, Vinall LE, Butterworth SL, Hardy RJ, Holloway J, Wadsworth ME et al. MUC7 haplotype analysis: results from a longitudinal birth cohort support protective effect of the MUC7*5 allele on respiratory function. *Ann Hum Genet* 2006;**70**:417–427.
29. Abecasis GR, Cookson WO. GOLD: graphical overview of linkage disequilibrium. *Bioinformatics* 2000;**16**:182–183.
30. Schneider S, Roessli D, Excoffier L. Arlequin ver. 2.000: a software for population genetics data analysis. Switzerland: Genetics and Biometry Laboratory, University of Geneva, 2000.
31. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* 2001;**68**:978–989.
32. Kabesch M. Gene by environment interactions and the development of asthma and allergy. *Toxicol Lett* 2006;**162**:43–48.
33. Shaheen SO, Sterne JA, Montgomery SM, Azima H. Birth weight, body mass index and asthma in young adults 5. *Thorax* 1999;**54**:396–402.
34. Chen Y, Rennie D, Cormier Y, Dosman J. Sex specificity of asthma associated with objectively measured body mass index and waist circumference: the Humboldt study. *Chest* 2005;**128**:3048–3054.
35. Jensen EJ, Pedersen B, Schmidt E, Dahl R. Serum IgE in nonatopic smokers, nonsmokers, and recent exsmokers: relation to lung function, airway symptoms, and atopic predisposition. *J Allergy Clin Immunol* 1992;**90**:224–229.
36. Tanabe T, Fujimoto K, Yasuo M, Tsushima K, Yoshida K, Ise H et al. Modulation of mucus production by interleukin-13 receptor alpha2 in the human airway epithelium. *Clin Exp Allergy* 2008;**38**:122–134.
37. Kuperman DA, Huang X, Koth LL, Chang GH, Dolganov GM, Zhu Z et al. Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma. *Nat Med* 2002;**8**:885–889.

Supplemental information on SNP methods

For Black et al: Contribution of functional variation in the *IL13* gene to allergy, hay fever and asthma in the NSHD longitudinal 1946 birth cohort

PCRs were conducted in 96 well plate format using approximately 20ng of buccal DNA in 10ul reactions.

IL13 rs20541 was typed using a tetra primer PCR method (Ye et al., 2001; Ye et al., 1992). This involved four oligonucleotide primers, the outer upper primer (5'-GGG ACT TCC GTG AGG ACT GAA T-3') and outer lower (5'-GCA CAG GCT GAG GTC TAA GCT AAG G-3') at 2 pmoles/ul; and the inner upper (5'- TAA AGA AAC TTT TTC GCG AGG GTC G-3') and inner lower (5'- ATG CTT TCG AAG TTT CAG TTG AGC T-3') at 10 pmoles/ul. The PCR reaction was carried out using Thermostart Taq polymerase, (Abgene), denaturation at 94°C for 15 mins followed by 35 PCR cycle of 30 secs denaturation, 30 secs annealing at 60°C, 30 secs extension at 72°C and 10 min final extension at 72°C. The control band (outer upper to outer lower primer) gives a product of 365 bp; the inner lower (A allele)-outer upper primer a product of 230 bp; the inner upper (G allele) -outer lower primer a product of 184 bp.

IL13 rs1800925 and *IL4* rs2070874 were incorporated into a 10ul multiplex (6 SNPs) PCR and primer extension, which used the *SNaPshot* procedure (ABI). The PCR contained 12 oligonucleotide primers, with concentrations adjusted to achieve equal amplification and 1 µl of Buffer IV (ABgene), 200 µM dNTPs and 0.05 µl of Taq polymerase (0.25 units, ABgene) with 95 °C initial denaturation for 5 min followed by 30/31 cycles of 30 sec denaturation at 95 °C, 30 sec at 61 °C and extension at 72 °C and a final 5 min extension at 72 °C. Excess primers and dNTPs from the multiplex PCR reactions were removed using 1.33 units of shrimp Alkaline Phosphatase and 0.8 units Exonuclease I added to 4 µl of PCR product at 37 °C for 60 min, then 75 °C for 15 min and cooled to 4 °C. For each sample, 1.67 µl of *SNaPshot*

Multiplex Ready Reaction Mix (Applied Biosystems) and 0.66 µl of primer mix containing six primers, of which, 0.2 µM of *PE IL13 rs1800925* primer (5'TTTTTTTTGCCTTTTCCTGCTCTTCCCTC 3') and 2.4 µM of *PE IL4 rs2070874* primer (5'GATATGCAGTGAGAATGTGAG 3 '), was added as a master mix to 1 µl of purified PCR product. The samples were incubated for 25 cycles at 96 °C for 10 sec, then 50 °C for 5 sec, then 60 °C for 30 sec and then cooled to 4°C. Post-extension treatment was performed by adding 1 µl of a master mix consisting of 0.33 µl of calf intestinal phosphatase (CIP) (1 unit/µl), 0.43 µl of 10X CIP buffer and 0.23 µl of dH₂O to each sample with 37 °C incubation for 60 min, followed by 75 °C for 15 min and a 4 °C hold.

2 µl of each sample was mixed with 10 µl of Hi-Di formamide and 0.25 µl of Genescan LIZ 120 size standard. The denatured and snap cooled samples were then run on an *ABI PRISM 3730XL Genetic Analyser* (ABI) using the built-in run module for *SNaPshot* products. *Genemapper version 4.0* (ABI) was used to analyse the data generated from the run. *Bins* for each marker were assigned. All traces were inspected by eye and typings checked.