Ann Allergy Asthma Immunol 110 (2013) 423–428

Contents lists available at SciVerse ScienceDirect

Genetic predictors of inflammation in the risk of occupational asthma in young apprentices

Dovi Stéphanie Acouetey, MD [*]; Denis Zmirou-Navier, MD, PhD [†]; Patrice Avogbe, PhD [*]; Paul Tossa, MD, PhD [*]; Thomas Rémen, PhD [*]; Annick Barbaud, MD, PhD [*]; José-Antonio Cornejo-Garcia, PhD [*,‡]; Miguel Blanca, MD [*,‡]; Abraham Bohadana, MD, PhD [*]; Christophe Paris, MD, PhD [*]; Jean-Louis Guéant, MD, PhD, AGAF [*]; and Rosa-Maria Guéant-Rodriguez, MD, PhD [*]

[*] *Nutrition, Genetics and Environment, INSERM-U954, Faculty of Medecine, Vandoeuvre lès Nancy, France*
[†] *Ecole des Hautes Etudes en Santé Publique, Rennes, France*
[‡] *Research Laboratory, Carlos Haya Hospital-Fundacion IMABIS, Malaga, Spain*

ARTICLE INFO

Article history:
Received for publication January 9, 2013.
Received in revised form March 31, 2013.
Accepted for publication April 3, 2013.

ABSTRACT

Background: The influence of genetic predictors of inflammation and atopy on occupational asthma in apprentices is not known.
Objectives: To assess the influence of genetic polymorphisms of *IL4RA*, *IL13*, *TNFA*, *IL1A*, and *IL5* on the decline of lung function and bronchial hyperresponsiveness in a prospective follow-up study of baker/pastry maker and hairdresser apprentices.
Methods: A total of 351 apprentices were included in the study. We performed skin testing, spirometry, fractional exhaled nitric oxide measurement, and methacholine hyperreactivity testing at the initial visit and during and at the end of the 18-month training period. Gene variants of *IL4RA, IL13, TNFA, IL1A*, and *IL5* were determined in DNA from nasal lavage.
Results: *IL13* R130Q/*IL4RA* S478P or *IL13 R130Q//IL4RA* Q551R were significant predictors of the decrease of forced expiratory volume and forced vital capacity ($P \leq .006$). Genotype GG of TNFAG308A was associated with bronchial hyperresponsiveness in the whole population and in nonatopic individuals (90.63% vs 9.38%; odds ratio, 3.78; 95% confidence interval, 1.10-12.83). *TNFA* GA and *IL5* CC and *TNFA* GA and *IL1A* CC were 2 epistatic predictors of exhaled nitrogen monoxide decrease during follow-up ($P = .02$ and $P = .004$, respectively). The association with *TNFA* GA and *IL1A* CC was the most significant in nonatopic bakers ($P < .001$).
Conclusion: We evidenced a predicting influence of *IL13/IL4RA* and *TNFA* in the early exposure to allergens and irritants that precedes occupational asthma. The significance of the associations in the absence of atopy suggests an influence of the genetics predictors related to inflammatory pathways.

Introduction

Apprentices represent a good model for studying the natural history of adult-onset occupational asthma (OA) and for describing the mechanisms associated with the incidence of airways inflammation and bronchial hyperresponsiveness (BHR). Several longitudinal studies have contributed to a better understanding of the clinical determinants of sensitization and inflammation in OA of apprentices exposed either to low-molecular-weight (LMW)[1] or high-molecular-weight (HMW)[2] agents. However, to date, none of these studies have evaluated the influence of gene polymorphisms, which may potentially predict airway inflammation.

The development of inflammation in response to early stage of exposure may enhance our understanding of the genetic factors that are involved in the mechanisms specifically related to the susceptibility of OA.[3] Moreover, there is a growing interest for taking into consideration a level of complexity that includes gene-gene and gene-environment interactions.[3] Among the high number of candidate genes, which have been evaluated as predictors of general asthma, some of them, *IL13*, *IL4RA, IL5, IL1A,* and *TNF* have been

Reprints: Rosa-Maria Guéant-Rodriguez, MD, PhD, Inserm U954, 9 rue de la Forêt de Haye, 54505 Vandoeuvre-Lès-Nancy, France; E-mail: Rosa-Maria.GueantRodriguez@medecine.uhp-nancy.fr.
Disclosures: Authors have nothing to disclose.
Funding Sources: This study was supported by grants from AFSSET (contract RD-2003-04), French Ministry of Labour (2002 Health and Occupation call for proposal), the regional Social Security office (CRAM Nord-Est), the Lorraine Region, ANR (the French National Research Agency; grant 05 9 75/ANR 05 SEST 021-01), PHRC (Hospital Clinical Research Programme, 2004), and INRS (National Institute for Labor Security). The Soufflet group and L'Oréal also provided financial support.

1081-1206/13/$36.00 - see front matter
http://dx.doi.org/10.1016/j.anai.2013.04.005

related to atopy and/or inflammation.[4] Beside their influence on atopy and inflammation, these candidate genes are also potent predictors of nonspecific BHR (NSBHR) in pediatric populations from Western Europe.[5] Some of these candidate genes have been previously studied in patients with OA caused by diisocyanates.[6] However, the association of these polymorphisms with the subsequent development of NSBHR has been scarcely investigated in apprentices.

In the present study, we aimed to assess the association between polymorphisms of *IL13* (R130Q rs20541), *IL4RA* (S478P rs1805015, and Q551R rs180275 variants), *IL-5* (C705T rs2069807), *IL1A* (C889T rs1800587), and *TNF* (G308A rs1800629) and the alteration of the lung function, incidence of NSBHR, and increase of fractional exhaled nitric oxide (FeNO). These lung parameters were chosen as indicators of airway inflammation in young bakery and hairdressing apprentices at high risk of OA. These two study groups of participants were exposed to contrasted types of occupational agents (HMW vs LMW).[7]

Methods

Study Participants and Design

Our ancillary study is based on a prospective follow-up study of 441 apprentices, including 209 baker/pastry maker apprentices and 142 hairdresser apprentices, in the Lorraine region, Northeastern France. The complete design has been described elsewhere.[8] The e-Methods provide additional detail. The study participants were visited in the beginning, during, and at the end of their 2-year training period. The participants included in our ancillary study were those seen at enrollment and at the last visit (n = 351, 80% of inclusions).

Examinations started within the 3 months of the beginning of the apprenticeship and were repeated every 6 months. Three follow-up visits were scheduled at 6, 12, and 15 months and a last visit after 2 years. All participants provided written consent, and the local ethic committee approved the study protocol. Participants were included provided they had not been previously exposed to substances known to induce OA, did not have physician-diagnosed asthma, and did not have a family history of atopy.

The research program has been authorized by the ethical committee of the University Hospital of Nancy (comité consultatif de protection des personnes dans la recherche biomédicale No. 02 09 02). Written consents were obtained from all participants or from their parents if the participants were minors. The database created for this project was declared to and approved by the French National Commission for Data Protection (commission nationale de l'informatique et des libertés No. 902 129).

Clinical Examination and Skin Prick Tests

A standardized questionnaire was used for personal, demographic information and for smoking habits. Personal history of atopy was assessed at the beginning and end of the study. It was defined by at least 1 positive skin prick test reaction to prevalent common aeroallergens. Briefly, the diameter of the wheal was considered positive when it was greater than 3 mm than the negative control (saline) in patients with a normal skin reactivity to histamine.

Fraction Exhaled Nitric Oxide

Immediately after the clinical examination, FeNO was measured in adherence with the American Thoracic Society and European Respiratory Society recommendations[9] and expressed in parts per billion. Measurements were performed using a chemiluminescence analyzer (NIOX 2.0 system; Aerocrine AB, Solna, Sweden). Evolution of FeNO was calculated as the difference between log FeNO values at the last visit and log FeNO values at first visit vs the duration of the follow up.

Spirometry Measurements and Airway Responsiveness to Methacholine

Spirometry was recorded for all study participants, and results were expressed as percentages of the predicted values given by the European Steel and Coal Community Working Party.[10] Forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), and maximal expiratory flow at various lung volumes were obtained by a forceful expiration after a maximum inspiratory maneuver. Three forced expiratory maneuvers, satisfying the recommended criteria,[3] were recorded as baseline measurement. The largest FVC and FEV_1 values were retained for analysis.

Apprentices also underwent methacholine challenge tests (MCTs), the results of which were considered positive if the FEV_1 decreased by at least 20% below the baseline value. The corresponding provocative concentration of methacholine (PC_{20}) was recorded. NSBHR incidence was defined according to the occurrence or aggravation of a positive MCT result[11] as follows: (1) occurrence of a positive response to the MTC at any visit in patients with a negative MCT result at inclusion; (2) decrease in the PC_{20} by at least one dose (using 3 cumulative successive doses of 0.5, 3.0, and 8.0 mmoL) in participants with a positive MCT result at the first visit; and (3) decrease of the normal dose response slope by 0.100 or more at any visit compared with the NDRS measured at the first visit in participants with at least a decrease of 15% of FEV_1. The 0.100 cutoff point corresponded to the mean decrease in all participants with positive MCT results. More details are given in the article by Tossa et al.[11]

DNA Extraction and Genotyping of Polymorphisms

DNA was extracted from 200 mL of nasal lavage using the QuiAmp mini kit (Quiagen, Courtaboeuf, France). The genotypes of the 4 polymorphisms were determined by real-time polymerase chain reaction (Roche Molecular Biochemicals, Lyon, France), as described (eMethods).[12,13]

Statistical Analysis

The statistical analyses were designed to explore the association between the selected polymorphisms and the variation of the indicators of airways inflammation during the study period, according to the apprenticeship training tracks. All analyses were performed with the STATA-11.1 SE statistical software package (StataCorp, College Station, Texas). Continuous variables were expressed as mean ± SD. FeNO was logarithmically transformed to normalize its skewed distribution. Polymorphisms were tested for Hardy-Weinberg equilibrium by using the χ^2 goodness-of-fit test. The genotype distribution among groups was compared by the Fisher exact test. The influence of genetic variants on functional parameters was evaluated at the different times of examination. Variation of the lung functions parameters was calculated by a variation index during the 2-year study period for each lung function parameter or FeNO. This variation index was defined as the difference between last determination and determination at enrollment vs follow-up duration. A 1-way analysis of variance was used to analyze the association of genotypes with independent variables. Only genotypes with association $P < .10$ were included in the multivariate analyses. These genotypes were adjusted for smoking status, training track, and atopy. Corrections for multiple testing among genotypes were made using the Bonferroni test. The follow-up of NSBHR according to exposure delay was analyzed by Kaplan-Meier analysis.

Results

Description of the Study Population

The demographic, clinical, and functional variables are summarized in Table 1; 47% of all apprentices were smokers, with no differences according to the exposure ($P = .35$). Incident NSBHR

Table 1
Characteristics of study participants at the first and last visits

Characteristic	No. (frequency) [95% CI] or mean ± SD			*P* value[a]
	All apprentices (n = 351)	Hairdressers (n = 142)	Bakers and pastry makers (n = 209)	
Age, y	16.9 ± 0.07	16.9 ± 0.11	16.9 ± 0.08	.79
Male sex	191 (0.54) [0.49-0.58]	10 (0.07, 0.03-0.11)	181 (0.87) [0.84-0.92]	<.001
Atopic status	115 (0.33) [0.28-0.38]	38 (0.27) [0.20-0.33]	77 (0.36) [0.30-0.41]	.02
Current smokers	64 (0.47) [0.41-0.51]	64 (0.45) [0.36-0.51]	100 (0.48) [0.42-0.54]	.35
FVC, % of predicted value				
First visit	91.27 ± 0.54	91.67 ± 0.83	91.01 ± 0.73	.13
Last visit	92.95 ± 0.87	90.54 ± 1.38	94.57 ± 0.73	.02
Variation index[b]	−0.24 ± 0.06	−0.45 ± 0.07	−0.09 ± 0.09	.007
FEV_1, % of predicted value				
First visit	92.37 ± 0.50	92.97 ± 0.69	92.00 ± 0.69	.35
Last visit	92.81 ± 0.81	91.32 ± 1.18	93.82 ± 1.08	.13
Variation index	−0.36 ± 0.05	−0.44 ± 0.06	−0.30 ± 0.07	.18
FEV_1/FVC, % of predicted value				
First visit	101.65 ± 0.40	101.87 ± 0.55	101.51 ± 0.50	.65
Last visit	100.24 ± 0.57	101.35 ± 0.88	99.49 ± 0.73	.08
Variation index	$-3.56.10^{-5} \pm 7.06.10^{-6}$	$-1.03.10^{-5} \pm 9.06.10^{-6}$	$-5.25.10^{-5} \pm 9.96.10^{-6}$	.003
FeNO, % of predicted value				
First visit	18.36 ± 0.9	15.07 ± 1.19	20.24 ± 1.26	.007
Last visit	19.03 ± 1.1	14.62 ± 1.04	22.23 ± 1.71	.003
Variation index	$1.33.10^{-4} \pm 1.17.10^{-4}$	$1.25.10^{-5} \pm 1.47.10^{-4}$	$2.12.10^{-3} \pm 1.64.10^{-4}$	.40
Incidence of NSBHR	71 (0.17) [0.14-0.21]	26 (0.16) [0.10-0.21]	45 (0.18) [0.13-0.22]	.59

Abbreviations: CI, confidence interval; FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; NSBHR, nonspecific bronchial hyperresponsiveness.
[a]Fisher exact test for categorical and analysis of variance for continuous variables.
[b]The variation index was calculated as the difference between log values at the last visit and log values at first visit divided by the duration of the follow-up.

was evidenced in 17.2% (n = 71) of the study participants during the follow-up period. NSBHR incidence was not different between the two groups of apprentices ($P = .59$). We quantified the exposure of apprentices to the main inhaled irritants and allergens, ammonia, hydrogen peroxide, persulfates. and flour dust (eTable 1 and eTable 2).

We observed a decrease of the lung function parameters related to NSBHR during the follow-up. The variation indexes of FVC, FEV_1, and FEV_1/FVC were reduced in 60.1%, 64.7%, and 58.5% of study participants, respectively. The index of FeNO increased in 53.4% of participants. The variation index of FVC was lower in the hairdresser apprentices compared with the pastry makers (−0.45 and −0.09, respectively, $P = .002$).

Influence of Polymorphisms on Variation of the Parameters of Airways Obstruction

The polymorphisms were in Hardy-Weinberg equilibrium. No significant difference was found in the distribution of polymorphisms and allele frequency according to the training tracks and NSBHR (eTable 3 and eTable 4).

A significant association was observed between T_H2-related polymorphisms (*IL13* R130Q, *IL4RA* S478P, and *IL4RA* Q551R) and the variation index of FEV_1 after 2 years of occupational exposure in univariate analysis (Table 2) and adjusted multivariate analysis (Table 3). Compared with the *RQQQ* genotype, the *RR* genotype of *IL13* rs20541 polymorphism predicted a lower FVC index (−0.36, $P = .02$) and a lower FEV_1 index (−0.34, $P = .008$) in all apprentices. The RR genotype predicted also a lower FVC index (eFig 1A) and a lower FEV_1 index (eFig 1B) in nonatopic bakers and pastry makers apprentices when compared with the RQ and QQ genotypes.

IL4RA S478P and *IL4RA* Q551P polymorphisms were predictors of lower FEV_1 among all apprentices (Table 2). The *SPPP* genotype predicted a lower FVC index in hairdressers (Table 3). The combination of *IL13* RR with either *IL4RA* 478 SPPP or *IL4RA* 551 *QRRR* predicted significantly a lower FVC index and a lower FEV_1 index, independently of training track, among all the epistatic interactions tested. The combination of *IL13 RR* with *IL4R* 551 *QRRR* genotypes was the most significant epistatic predictor of lower FVC index and FEV_1 index in nonatopic apprentices (eFig 1C and D, and Table 3).

Table 2
Association of gene variants and significant gene-gene combination of variants with the variation of the parameters of airway inflammation during the study period (univariate analysis) in all 351 apprentices

Gene	Polymorphisms	Genotype	FVC index,[a] mean ± SD		*P* value[b]	FEV_1 index,[a] mean ± SD		*P* value[b]
			With genotype	Without genotype		With genotype	Without genotype	
IL4RA S478P	rs1805015	SS (n = 333)	−0.24 ± 1.13	0.53 ± 1.16	.06	−0.34 ± 0.94	−0.61 ± 0.94	.03
IL4RA Q551R	rs180275	QQ (n = 338)	−0.24 ± 1.15	−0.41 ± 1.21	.22	−0.31 ± 0.92	−0.56 ± 0.99	.03
IL13 R130Q	rs20541	RR (n = 284)	−0.44 ± 1.16	−0.09 ± 1.09	.02	−0.52 ± 0.89	−0.20 ± 1.06	.01
TNFA -308	rs1800629	GG (n = 351)	−0.32 ± 1.25	−0.24 ± 0.97	.56	−0.42 ± 1.02	−0.31 ± 0.75	.37
IL1A -889	rs1800587	CC (n = 351)	−0.36 ± 1.13	−0.29 ± 1.24	.60	−0.45 ± 1.01	−0.37 ± 0.91	.47
IL5 -703	rs2069821	CC (n = 351)	−0.37 ± 1.09	−0.24 ± 1.25	.32	−0.44 ± 0.93	−0.35 ± 0.99	.44
IL13 RQQQ *IL4RA* 478 SS	rs20541/rs1805015	RQQQ/SS	0.04 ± 1.07	−0.46 ± 1.15	.006	−0.10 ± 0.95	−0.53 ± 0.93	.003
IL13 RQQQ/*IL4RA* 551 QQ	rs20541/rs180275	RQQQ/QQ	0.06 ± 1.09	−0.44 ± 1.14	.005	−0.10 ± 0.95	−0.50 ± 0.94	.006
TNFA GA/ *IL5* TT	rs1800629/rs2069821	GA/TT	−0.51 ± 0.70	−0.29 ± 1.23	.23	−0.38 ± 0.60	0.39 ± 1.01	.96

Abbreviations: FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity.
[a]The variation index of parameters of airway inflammation measures the variation of each parameter between the inclusion time and the last determination using the formula (last determination − determination at enrollment) vs (follow-up duration).
[b]One-way analysis of variance.

Table 3
Association between polymorphisms and the variation of lung function during the study period, according to the training track and the atopic status (multivariate analysis)

Variable	FVC index[a]		FEV_1 index[a]		FEV_1/FVC index[a]	
	β (SE)[b]	*P* value[c]	β (SE)[b]	*P* value[c]	β (SE)[b]	*P* value[c]
All Apprentices						
IL4RA 478 SPPP vs SS (n = 333)	−0.26 (0.15)	.10	−0.26 (0.13)	.04	$-6.72.10^{-6}$ ($1.85.10^{-5}$)	.72
IL4R576 QRRR vs QQ (n = 338)	−0.15 (0.14)	.21	−0.21 (0.11)	.06	$-2.03.10^{-5}$ ($1.68.10^{-5}$)	.23
IL13 RR vs RQQQ (n = 284)	−0.36 (0.15)	.02	−0.34 (0.13)	.008	$-3.33.10^{-5}$ ($1.45.10^{-4}$)	.60
IL13 RR/*IL4RA* 478 SPPP	−0.51 (0.16)	.002	−0.44 (0.13)	.001	$-6.41.10^{-6}$ ($2.10.10^{-5}$)	.75
IL13 RR/*IL4RA* 551 QRRR	−0.50 (0.17)	.004	−0.42 (0.14)	.004	$1.14.10^{-6}$ ($2.20.10^{-5}$)	.95
Hairdressers						
IL4RA 478 SPPP vs SS (n = 131)	−0.40 (0.18)	.03	−0.30 (0.15)	.05	$9.14.10^{-6}$ ($2.112.10^{-5}$)	.67
Bakers and Pastry Makers						
IL4R576 QRRR vs QQ (n = 203)	−0.15 (0.21)	.47	−0.32 (0.17)	.06	−1.94.658.10−5 ($2.51.10^{-5}$)	.07
All Nonatopic Apprentices						
IL13 RR vs RQQQ (n = 189)	−0.35 (0.17)	.04	−0.28 (0.15)	.06	$-2.27.10^{-6}$ ($2.21.10^{-5}$)	.92
IL13 RR/*IL4RA* 478 SPPP	−0.52 (0.18)	<.001	−0.40 (0.16)	.01	$3.33.10^{-6}$ ($2.39.10^{-5}$)	.89
IL13 RR/*IL4RA* 551 QRRR	−0.54 (0.19)	<.001	−0.38 (0.17)	.02	$1.58.10^{-5}$ ($2.54.10^{-5}$)	.54

[a]The variation index of parameters of airway inflammation measures the variation of each parameter between the inclusion time and the last determination using the formula (last determination − determination at enrollment) vs (follow-up duration).
[b]Adjusted for smoking status, training track, and atopic status.
[c]*P* indicates the significance of the contribution of the genotype to the model (see e-Table 5 for univariate analysis).

Polymorphisms Involved in NSBHR and FeNO

The genotype *TNFA* GG was more frequent in participants with NSBHR (18.8% vs 10.9%, $P = .05$). The association remained significant in nonatopic participants but not in those with atopy (Table 4). We observed no association of genetic determinants with the variation of FeNO levels during follow-up in the whole population (eTable 5). After stratification according to atopic status, 2 epistatic interactions, *TNFA* GA *and IL5* CC and *TNFA* GA and *IL1A* CC were associated with decrease of FeNO in all nonatopic apprentices (Table 4). *TNFA* GA and *IL1A* CC were predictors of reduction of FeNO in nonatopic bakers ($P < .001$). On the other hand, *TNFA* GG and *IL1A* CC were associated with an increase of FeNO among hairdressers ($P = .04$).

Follow-up of NSBHR

The cumulative incidences of NSBHR according to *TNFA* GG vs *GAAA* genotypes at 24 months were 19.6% and 6.4%, respectively, in atopic participants (Fig 1A) and 28.5% and 8.5%, respectively, in nonatopic participants (Fig 1B).

Discussion

The decrease in lung function depends on complex mechanisms that involve age, genetic predictors, smoking habits, and environmental exposure.[14] Several longitudinal studies on apprentices exposed either to LMW[1] or HMW[15] agents have contributed to a better understanding of the clinical determinants of occupational sensitization and inflammation in OA. However, to date none of these studies have considered the influence of gene polymorphisms on lung function and airway inflammation assessed by NSBHR and measurement of FeNO.

Previous population studies had reported their association of *IL13* and *IL4 RA* with atopy and asthma in children.[16] Consistently, *IL4RA* S478P and Q551R were associated with a decrease in FEV_1 and FVC after 2 years of exposure (Table 2 and Table 3). The receptor of *IL4* triggers the effects of interleukin (IL) 13 and IL-14 in allergy-related inflammation by promoting T_H2 cell differentiation and IgE synthesis.[17] In our study, both *IL4RA* S478P and *IL4RA* Q551R predicted the risk of decreased lung function. The associations may depended on the type of exposure.[18] The Q551R polymorphism is located in a region that encodes a STAT6 recruiting domain of *IL4RA*, making its influence possible through this pathway.[19]

In our study, *IL13* R130Q was significantly associated with FVC index and FEV_1 index (Table 3). The most frequent homozygous genotype predicted a faster decline of lung function (eFig 1A and B). This polymorphism encodes an amino acid residue, which is located within the D helix, close to the C-terminal region of IL-13.[20] IL-13 is a cytokine secreted by many cell types, including T_H2 cells.[21] It has effects that are related with OA, including airway hyperresponsiveness, goblet cell metaplasia, and mucous hypersecretion, which all contribute to airway obstruction.[22] Because IL-13 is a ligand of the *IL4RA* subunit, it is thus possible that the R130Q polymorphism influences the interaction between D helix and the *IL4RA* subunit. We previously found a gene-gene interaction of *IL13* and *IL4RA* polymorphisms on the risk of β-lactam allergy.[19] Currently, the combination of *IL13* RR and *IL4RA* 551 QRRR genotypes predicted a faster decrease in lung function, regardless of the type of occupational exposure (eFig 1C and D). The underlying molecular mechanisms of this association need to be clarified because the computer modeling of the *IL13/IL4Ra* interaction suggests that the arginine of the 130RR variant, but not the glutamic acid of the less frequent variant, repulses the histidine 131 of *IL4RA*.[16] The association remained significant in nonatopic apprentices after stratification. This finding suggests that the underlying mechanism is related to downstream signaling pathways of inflammation rather than to IgE production. Similarly, these combined genotypes were significantly associated with diisocyanate-induced asthma in a previous study on exposed workers.[21] These genes are associated with asthma susceptibility by regulating the differentiation of naive CD41-T_H cells into a T_H2 cell phenotype.[18]

Regarding phenotypes of asthma, we found that the *TNFA* -G308A polymorphism was associated with NSBHR in nonatopic apprentices (Table 4 and Fig 1B). The *TNFA* -G308GA polymorphism is located in the promoter region of *TNFA* and has been associated with increased secretion and promoter activity of *TNFA*.[22] In the European Community Health Respiratory Survey, Castro-Giner et al[23] reported a mild association of the *TNFA* -G308A polymorphism with asthma and NSBHR. Another study found no association between *TNFA* -G308A and asthma in atopic individuals.[24] The role of *TNFA* polymorphism in airway inflammation of asthmatic patients is supported by its influence on increased expression of tumor necrosis factor.[25] Moreover, *TNFA* enhances NSBHR in healthy indivdiuals[26] and asthmatic patients.[25] The relation between *TNFA* and OA has been clearly evidenced in animal models of by toluene diisocyanate–induced asthma.[27] In contrast to these experimental-based findings, little is known about the influence of *TNFA* polymorphisms in OA, with one case-control study reporting no association between the *TNFA* -G308A

Table 4
Association between genetic polymorphisms and/or gene-gene interactions with the incidence of NSBHR and the variation of FeNO during the study period (multivariate analysis)

Variable	NSBHR		FeNO index	
	OR (95% CI)[a]	*P* value[b]	β (SE)[c]	*P* value[b]
All Nonatopic Apprentices (N = 236)				
TNFA -308 GG	3.74 (1.10-12.82)	.04	$3.99.10^{-4}$ ($5.14.10^{-4}$)	.27
TNF GG / IL1A CC	2.25 (1.01-5.01)	.05	$3.02.10^{-4}$ ($3.55.10^{-4}$)	.40
TNFA GA/IL5 CC	2.49 (0.14-43.45)	.53	$-2.61.10^{-3}$ ($1.09.10^{-3}$)	.02
TNFA GA/IL1A CC	0.46 (0.10-2.07)	.32	$-1.42.10^{-3}$ ($4.89.10^{-3}$)	.004
Nonatopic Bakers and Pastry Makers (n = 132)				
TNFA GG/IL5 TT	3.76 (1.11-12.74)	.06	$1.69.10^{-4}$ ($5.73.10^{-4}$)	.77
TNFA GA/IL5 CC	NA		-0.02 ($2.54.10^{-3}$)	<.001
TNFA GA / IL1A CC	0.48 (0.06-4.21)	.51	$-1.95.10^{-3}$ ($8.44.10^{-4}$)	.02
Nonatopic Hairdressers (n = 104)				
TNFA GG/IL1A TT	5.75 (1.03-32.08)	.05	$-7.10.10^{-4}$ ($6.39.10^{-4}$)	.27
TNFA GG/IL1A CC	2.40 (0.78-7.33)	.12	$6.61.10^{-4}$ ($3.09.10^{-4}$)	.04

Abbreviations: CI, confidence interval; FeNO, fractional exhaled nitric oxide; NSBHR, nonspecific bronchial hyperresponsiveness; OR, odds ratio.
[a]Adjusted ORs using logistic regression models.
[b]*P* indicates the significance of the contribution of the genotype to the model.
[c]Adjusted for smoking status, training track, and atopic status.

polymorphism and toluene diisocyanate–induced asthma in 142 patients and 45 controls.[28] However, the atopic status was not considered in this study. Interestingly, we reported an association between the *TNFA* GG polymorphism and the incidence of NSBHR in a cohort of young apprentices (n = 351), which remained significant in nonatopic individuals (n = 281). A similar result was found in a case-control study conducted in the general Chinese population.[29] Currently, our cohort allowed us to explore the incidence of NSBHR in apprentices since the very beginning of exposure to occupational allergens. This study design is more accurate to evaluate the predictive value of *TNFA* in OA than comparing asthmatic patients with the general population.

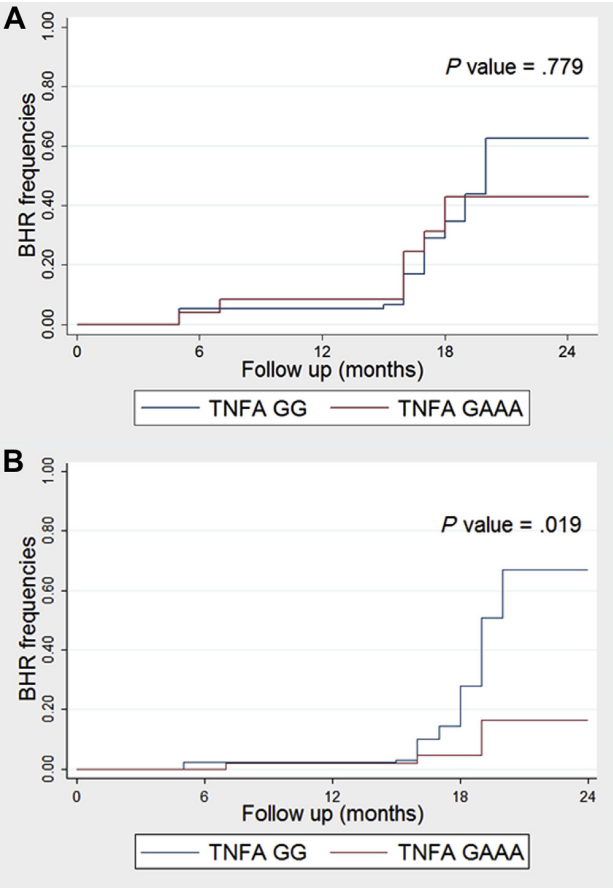


Figure 1. Cumulative incidence proportion of nonspecific bronchial hyperresponsiveness (NSBHR) according to the *TNFA* GG and GAAA genotypes at 24 months (log-rank test) among atopic *(A)* and nonatopic *(B)* apprentices. *TNFA* GG predicted a higher cumulative incidence of NSBHR at 24 months compared with the GAAA genotype in nonatopic apprentices.

IL-1 seems to play a significant role in OA in regards to experimental models.[30,31] Antibody neutralization of *IL1A* prevented the production of IgG_1 after toluene diisocyanate challenge in mice,[31] whereas toluene diisocyanate challenge in sensitized mice failed to increase *IL4* expression in *IL11R* knockout mice. In contrast, population-based evidence is lacking. This association could be related to atopy, as previously reported in nonasthmatic individuals.[32] We have previously shown that the incidence of NSBHR is associated with the sensitization to flour.[10] In contrast, we observed an epistatic influence of *IL1A* and *TNFA* polymorphisms on variation of lung function, NSBHR incidence, and variation of FeNO during the study period in nonatopic individuals (Tables 3 and 4). This finding suggests that the 2 cytokines act synergistically, a hypothesis that needs to be studied at the experimental level.

We also observed that some polymorphisms were associated with the variation of FeNO levels among nonatopic apprentices (Table 4). *TNFA* GA and *IL5* CC and *TNFA* GA and *IL1A* CC were 2 epistatic predictors of a FeNO decrease during follow-up, an association stronger among nonatopic bakers and pastry makers. The *TNFA* GG and *IL1A* CC genotype showed an increase of FeNO during the follow-up of nonatopic hairdressers ($P = .04$). This finding is consistent with the influence of inflammatory cytokines, such as tumor necrosis factor and T_H1 cytokines, on FeNO through expression of inducible nitric oxide synthase.[33]

Our study has some limitations. First, the number of participants agrees with the study power estimate, but a replication is needed to reach a definitive conclusion. Second, interpretation of our results was limited by the impossibility of evaluating qualitatively and quantitatively the exposure and IgE response against allergens.[34]

The strength of our study was to evaluate the occurrence of NSBHR and airway inflammation in 2 young adult groups since the very beginning of exposure to occupational allergens. Each participant was considered his/her own control, comparing the basal and final characteristics after a definite period of exposure. Our findings support the hypothesis of the existence of gene-environment and gene-gene interactions in occupational NSBHR, in agreement with the available knowledge on nonoccupational settings.

This study evidenced the predicting influence of variants of *IL4RA, IL13, TNFA, IL1A,* and *IL5* on NSBHR and airway inflammation since the very beginning of exposure to an occupational environment in young adult apprentices. Most of the associations remained significant in absence of atopy, suggesting that inflammatory downstream signaling pathways of these genetic predictors are involved in the early step that precedes OA.

Acknowledgments

We thank the directors and teachers of the 6 apprenticeship schools of Lorraine. The authors are indebted to Denise Tramoy and René Debard for their excellent technical contribution in the genotyping and to Paloma Campo (Malaga, Spain) for her help in reading the draft of our manuscript.

Supplementary Data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.anai.2013.04.005

References

[1] Dragos M, Jones M, Malo JL, Ghezzo H, Gautrin D. Specific antibodies to diisocyanate and work-related respiratory symptoms in apprentice car-painters. *Occup Environ Med*. 2009;66:227–234.

[2] Gautrin D, Ghezzo H, Infante-Rivard C, Malo JL. Incidence and host determinants of work-related rhinoconjunctivitis in apprentice pastry-makers. *Allergy*. 2002;57:913–918.

[3] Christiani DC, Mehta AJ, Yu CL. Genetic susceptibility to occupational exposures. *Occup Environ Med*. 2008;65:430–436.

[4] Hoffjan S, Nicolae D, Ober C. Association studies for asthma and atopic diseases: a comprehensive review of the literature. *Respir Res*. 2003;4:4–14.

[5] Liu X, Beaty TH, Deindl P, et al. Associations between specific serum IgE response and 6 variants within the genes IL4, IL13, and IL4RA in German children: the German Multicenter Atopy Study. *J Allergy Clin Immunol*. 2004; 113:489–495.

[6] Bernstein DI, Wang N, Campo P, et al. Diisocyanate asthma and gene-environment interactions with IL4RA, CD-14, and IL-13 genes. *Ann Allergy Asthma Immunol*. 2006;97:800–806.

[7] Mounier-Geyssant E, Barthelemy JF, Mouchot L, Paris C, Zmirou-Navier D. Exposure of bakery and pastry apprentices to airborne flour dust using pm2.5 and pm10 personal samplers. *BMC Public Health*. 2007;7:311. http://dx.doi.org/10.1186/1471-2458-7-311.

[8] Tossa P, Bohadana A, Demange V, et al. Early markers of airways inflammation and occupational asthma: rationale, study design and follow-up rates among bakery, pastry and hairdressing apprentices. *BMC Public Health*. 2009;9:113. http://dx.doi.org/10.1186/1471-2458-9-113.

[9] ATS/ERS. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med*. 2005;171:912–930.

[10] Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows: report of the Working Party Standardization of Lung Function Tests, European Community for Steel and Coal: Official Statement of the European Respiratory Society. *Eur Respir J Suppl*. 1993;16:5–40.

[11] Tossa P, Paris C, Zmirou-Navier D, et al. Increase in exhaled nitric oxide is associated with bronchial hyperresponsiveness among apprentices. *Am J Respir Crit Care Med*. 2010;182:738–744.

[12] Bosco P, Gueant-Rodriguez RM, Anello G, et al. Association of IL-1 RN*2 allele and methionine synthase 2756 AA genotype with dementia severity of sporadic Alzheimer's disease. *J Neurol Neurosurg Psychiatry*. 2004;75: 1036–1038.

[13] Gueant-Rodriguez RM, Gueant JL, Viola M, Tramoy D, Gaeta F, Romano A. Association of tumor necrosis factor-alpha -308G>A polymorphism with IgE-mediated allergy to betalactams in an Italian population. *Pharmacogenomics J*. 2008;8:162–168.

[14] Kohansal R, Martinez-Camblor P, Agusti A, Buist AS, Mannino DM, Soriano JB. The natural history of chronic airflow obstruction revisited: an analysis of the Framingham offspring cohort. *Am J Respir Crit Care Med*. 2009;180:3–10.

[15] Gautrin D, Infante-Rivard C, Ghezzo H, Malo JL. Incidence and host determinants of probable occupational asthma in apprentices exposed to laboratory animals. *Am J Respir Crit Care Med*. 2001;163:899–904.

[16] Heinzmann A, Mao XQ, Akaiwa M, Kreomer RT, et al. Genetic variants of IL-13 signalling and human asthma and atopy. *Hum Mol Genet*. 2000;9: 549–559.

[17] Isidoro-Garcia M, Davila I, Laffond E, Moreno E, Lorente F, Gonzalez-Sarmiento R. Interleukin-4 (IL4) and interleukin-4 receptor (IL4RA) polymorphisms in asthma: a case control study. *Clin Mol Allergy*. 2005;3:15.

[18] Maestrelli P, Boschetto P, Fabbri LM, Mapp CE. Mechanisms of occupational asthma. *J Allergy Clin Immunol*. 2009;123:531–542.

[19] Gueant-Rodriguez RM, Romano A, Beri-Dexheimer M, Viola M, Gaeta F, Gueant JL. Gene-gene interactions of IL13 and IL4RA variants in immediate allergic reactions to betalactam antibiotics. *Pharmacogenet Genomics*. 2006; 16:713–719.

[20] Zurawski SM, Vega F Jr, Huyghe B, Zurawski G. Receptors for interleukin-13 and interleukin-4 are complex and share a novel component that functions in signal transduction. *EMBO J*. 1993;12:2663–2670.

[21] Kips JC. Cytokines in asthma. *Eur Respir J Suppl*. 2001;34:24s–33s.

[22] Elahi MM, Asotra K, Matata BM, Mastana SS. Tumour necrosis factor alpha-308 gene locus promoter polymorphism: an analysis of association with health and disease. *Biochim Biophys Acta*. 2009;1792:163–172.

[23] Castro-Giner F, Kogevinas M, Machler M, et al. TNFA -308G>A in two international population-based cohorts and risk of asthma. *Eur Respir J*. 2008;32: 350–361.

[24] Louis R, Leyder E, Malaise M, Bartsch P, Louis E. Lack of association between adult asthma and the tumour necrosis factor alpha-308 polymorphism gene. *Eur Respir J*. 2000;16:604–608.

[25] Commins SP, Borish L, Steinke JW. Immunologic messenger molecules: cytokines, interferons, and chemokines. *J Allergy Clin Immunol*. 2010;125:53–72.

[26] Thomas PS, Yates DH, Barnes PJ. Tumor necrosis factor-alpha increases airway responsiveness and sputum neutrophilia in normal human subjects. *Am J Respir Crit Care Med*. 1995;152:76–80.

[27] Matheson JM, Lemus R, Lange RW, Karol MH, Luster MI. Role of tumor necrosis factor in toluene diisocyanate asthma. *Am J Respir Cell Mol Biol*. 2002; 27:396–405.

[28] Beghe B, Padoan M, Moss CT, et al. Lack of association of HLA class I genes and TNF alpha-308 polymorphism in toluene diisocyanate-induced asthma. *Allergy*. 2004;59:61–64.

[29] Shin HD, Park BL, Kim LH, et al. Association of tumor necrosis factor polymorphisms with asthma and serum total IgE. *Hum Mol Genet*. 2004;13: 397–403.

[30] Whelan R, Kim C, Chen M, Leiter J, Grunstein MM, Hakonarson H. Role and regulation of interleukin-1 molecules in pro-asthmatic sensitised airway smooth muscle. *Eur Respir J*. 2004;24:559–567.

[31] Johnson VJ, Yucesoy B, Luster MI. Prevention of IL-1 signaling attenuates airwayhyperresponsiveness and inflammation in a murine model of toluene diisocyanate-induced asthma. *J Allergy Clin Immunol*. 2005;116: 851–858.

[32] Karjalainen J, Hulkkonen J, Pessi T, et al. The IL1A genotype associates with atopy in nonasthmatic adults. *J Allergy Clin Immunol*. 2002;110:429–434.

[33] Ricciardolo FL. Multiple roles of nitric oxide in the airways. *Thorax*. 2003;58: 175–182.

[34] Aalto-Korte K, Makinen-Kiljunen S. Specific immunoglobulin E in patients with immediate persulfate hypersensitivity. *Contact Dermatitis*. 2003;49:22–25.

eMethods

Skin Prick Tests (SPTs)

Atopy assessed at entry and the end of the study was defined by positive skin reaction to common aeroallergens including dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), animal danders (cat, dog), pollens (mixtures of the following pollens: grass, weed, Fagacae, Betulaceae, cereals) and molds (*Alternaria tenius* and *Aspergillus fumigatus*). All extracts were commercialized by Allerbio-ALK laboratory (Varennes en Argonnes, France). Prick tests were performed on the forearm. The diameter of the wheal was measured at 20 minutes then compared with those of negative (saline) and positive (histamine) controls. It was considered positive when the diameter was equal or more than those of the negative control and if the patient had normal skin reactivity to histamine. The diameter of the wheal was considered as positive when it was greater than 3 mm that of negative controls (saline) in patients with a normal skin reactivity to histamine.

Fraction Exhaled Nitric Oxide (FeNO)

Measurements were taken by a trained technician using a chemiluminescence analyzer (NIOX 2.0 system; Aerocrine AB, Solna, Sweden). Three correctly performed exhalations were recorded during each session. Any exhalation not meeting American Thoracic Society and European Respiratory Society requirements was rejected by the NIOX system. In particular, subjects with a recent respiratory infection were excluded from the FeNO tests.

Airways Responsiveness to Methacholine

Nonspecific airway responsiveness was evaluated using the methacholine challenge test (MCT) using 3 cumulative successive doses (0.5, 3.0, and 8.0 μmoL) and bronchial hyperresponsiveness incidence, defined according of the occurrence or aggravation of a positive MTC result as follows [1,5]: (1) occurrence of a positive response to the MCT at any visit in patients with a negative MCT result at inclusion; (2) aggravation of the provocative concentration of inhaled methacholine (PC_{20}) required to reduce forced expiratory volume in 1 second (FEV_1) by 20%, at least for one lower dose in subjects with positive MCT results at the first visit. 3. Aggravation of the coefficient of the normal dose response slope with a decrease by 0.100 or more at any visit compared with the normal dose response slope measured at the first visit in subjects with at least a decrease of 15% of FEV_1 whatever the visit, (the 0.100 cutoff point corresponding to the mean decrease in all patients with positive MCT results with a significant PC_{20}).

Nasal Lavage Technique and DNA Extraction From Collected Nasal Cells

The nasal lavage (NL) was adapted from the Hilding procedure and performed with the subject in a sitting position.[1] DNA was extracted from 200 mL of NL using the QuiAmp mini kit (Quiagen, Courtaboeuf, France). The genotypes of the 4 polymorphisms were determined by real-time polymerase chain reaction (PCR) (Roche Molecular Biochemicals, Lyon, France). The anchor (Flu) and detection probes (LC) were labeled at the 30 ends with fluorescein and at the 50 ends with LC-Red-640. They covered the mutation sites and were modified at the 30 ends by phosphorylation to avoid extension (Tib MolBiol Syntheselabor GmbH, Berlin, Germany). The pair of probes hybridized template DNA with a 1-bp gap between them. PCR was performed in a 10-mL mixture in the Light-Cycler glass capillaries. The PCR primers, Flu, and LC primers were as follows, respectively: 5′AGCATGTCCGAGACAC3', 5′CTTCCCGCCTAC CCAA3′, 5′CCTGTCTCTCTGCAAATAATGTGCTTTCGT3′, and 5′GTTTCA GTTGACCGTC CCT3′ for *IL13* gene (rs20541); 5′GAGCCAAGTCC TCCTG3′, 5′CTCGGGTTCTACTTCCT3′, 5′AGCTTCAGCAACCCCCT3′, and 5′GCCAGTCACCGTGTCCCAG3′ for the rs1805015 variant of the *IL4RA* gene; and 5′AGCTCTCTGAGCCAAC3′, 5′CTTGTAACCAGCCT CTCC3′, 5′ACCCTGCTCCACCGCAT3′, and 5′ACAAACTCCCGATAGCCA CTG3′ for the rs180275 variant of the *IL4RA* gene; 5′TGTGACCCTTGT CAGAAAGAG3′, 5′TGAGGTCTCAAGATGATGTXTCAG3′, and 5′GAAC AGAATACATACAGATCCAGGAGT3′ for the rs2069807 variant of *IL5*; 5′CCTGCATCCTGTCTGGAAGTTA3′, 5′CTGCACCTTCTGTCTCGGTTT3′, and 5′AACCCCGTCCCCATGCCCC3′, 5′CAAAACCTATTGCCTCCATTTC TTTTGGGGAC3′ for the rs1800629 variant of *TNFA* gene; 5′TGTT CTACCACCTGAACTAGGC3′, 5′TGGCTAAGTTTGGGAATGGAGAT3′, and 5′GGAAGGCATGGATTTTTACATATGAGCC3′, 5′TTATTATCATTAGTCCGT TGTAGTAACT3′ for the rs1800587 variant of *IL1A*. The details of determinations are described previously.[2,3]

eTable 1
Exposure of hairdresser apprentices for inhalated irritants, ammonia, hydrogen peroxide, and persulfates[a]

	Hydrogen peroxide	Ammonia	Persulfates
Personal exposure			
No. of samples	39	53	52
Arithmetic mean ± SD	0.05 ± 0.04	0.90 ± 0.76	0.02 ± 0.02
Range, mg/m^3	0.003-0.18	0.02-4.49	0.001-0.12
Technical area in the customer salon (permanenting, dyeing, bleaching with the customers)			
No. of samples	53	52	52
Arithmetic mean ± SD	0.04 ± 0.03	0.72 ± 0.49	0.02 ± 0,02
Range, mg/m^3	0.003-0.18	0.13-3.03	0.00025-0.1
Shampoo area			
No. of samples	51	51	51
Arithmetic mean ± SD	0.03 ± 0.03	0.65 ± 0.40	0.02 ± 0,02
Range, mg/m^3	0.003-0.13	0.09-2.39	0.00025-0.13
Technical room (mixing the chemicals for permanenting, dyeing, and bleaching)			
No. of samples	52	52	52
Arithmetic mean ± SD	0.04 ± 0.05	0.66 ± 0.45	0.02 ± 0,02
Range, mg/m^3	0.003-0.34	0.13-2.64	0.002-0.08
Mean ambient air concentrations in each salon	53	52	52
No. of samples	53	52	52
Arithmetic mean ± SD	0.04 ± 0.03	0.68 ± 0.42	0.02 ± 0,02
Range, mg/m^3	0.003-0.15	0.13-2.69	0.002-0.08

[a]Adapted from Mounier-Geyssant et al.[4]

eTable 2
Exposure of baker and pastry maker apprentices to flour dust[a]

	$PM_{2.5}$		PM_{10}	
	Summer	Winter	Summer	Winter
Baker apprentices				
No. of samples	17	21	15	20
Arithmetic mean ± SD	0.50 ± 0.37	0.71 ± 0.37	0.63 ± 0.36	1.10 ± 0.83
Range, mg/m^3	0.17-1.52	0.19-1.42	0.17-1.73	0.28-4.04
Pastry apprentices				
No. of samples	4	13	3	13
Arithmetic mean ± SD	0.29 ± 0.06	0.35 ± 0.17	0.47 ± 0.13	0.44 ± 0.16
Range, mg/m^3	0.22-0.36	0.20-0.70	0.33-0.58	0.22-0.82

Abbreviation: PM, particulate matter.
[a]Adapted from Mounier-Geyssant et al.[5]

eTable 3
Distribution of T_H2 genetics polymorphisms and allele frequencies among patients with BHR in the study population according to the training track

	Bakers and pastry makers with BHR		Hairdressers with BHR		*P* value[a]	All apprentices with BHR		*P* value[a]
	No.	% [95% CI]	No.	% [95% CI]		No.	% [95% CI]	
IL4RA S478P (rs1805015)								
SS	156	78.0 [72.1-83.8]	97	74.4 [67.0-81.9]	.75	38	74.5 [59.7-83.2]	.79
SP	31	15.5 [10.4-20.5]	24	18.0 [11.5-24.6]		10	19.6 [10.8-32.0]	
PP	13	06.5 [03.1-10.0]	10	07.5 [03.0-12.0]		3	05.9 [02.1-15.7]	
Allele S	343	85.8 [82.0-89.0]	222	83.3 [78.5-87.4]	.42	86	84.3 [76.0-90.1]	.87
Allele P	57	14.2 [11.2-18.0]	44	16.5 [12.6-21.5]		16	15.7 [09.9-24.0]	
IL4RA Q551R (rs180275)								
QQ	133	65.5 [58.9-72.1]	86	63.7 [55.5-71.9]	.61	36	66.67 [53.3-77.8]	.65
QR	57	28.1 [21.8-34.3]	43	31.9 [23.9-39.8]		14	25.93 [16.1-39.0]	
RR	13	06.4 [03.0-09.8]	6	04.4 [00.9-07.9]		4	07.41 [03.0-17.6]	
Allele Q	266	91.1 [75.4-83.2]	172	93.5 [74.4-84.0]	.35	72	90.00 [81.5-94.8]	.47
Allele R	26	08.9 [16.8-24.6]	12	06.5 [16.0-25.6]		8	10.00 [05.2-18.5]	
IL13 R130Q (rs20541)								
RR	110	65.5 [58.2-72.7]	84	72.4 [64.2-80.6]	.47	31	68.89 [54.3-80.5]	.92
RQ	47	28.0 [21.1-34.8]	26	22.4 [14.8-30.1]		11	24.44 [14.3-38.8]	
QQ	11	06.5 [2.8-10.31]	6	05.2 [11.1-09.2]		3	06.67 [02.4-17.9]	
Allele R	267	79.5 [74.8-83.4]	194	83.6 [78.3-87.8]	.21	73	81.11 [71.8-87.8]	.99
Allele Q	69	20.5 [16.6-25.2]	38	16.4 [12.2-21.7]		17	18.89 [12.8-28.2]	
IL5 -703 (rs2069821)								
CC	102	46.4 [39.7-53.0]	73	48.7 [40.6-56.7]	.69	28	44.4 [32.8-56.7]	.48
CT	97	44.1 [37.5-50.7]	60	40.0 [32.1-47.9]		26	41.3 [29.9-53.6]	
TT	22	09.5 [05.6-13.4]	17	11.3 [06.2-16.4]		9	14.3 [05.5-23.0]	
Allele C	301	68.9 [64.4-73.0]	206	68.9 [63.4-73.9]	.10	82	65.6 [56.9-73.4]	.38
Allele T	136	31.1 [27.0-35.6]	93	31.1 [26.1-36.6]		43	34.4 [26.7-43.1]	

Abbreviations: CI, confidence interval; BHR, bronchial hyperresponsiveness.
[a]Fisher exact test for categorical variables, with Bonferroni correction for multiple testing.

eTable 4
Distribution of T_H1 genetics polymorphisms and allele frequencies among patients with BHR in the study population according to the training track

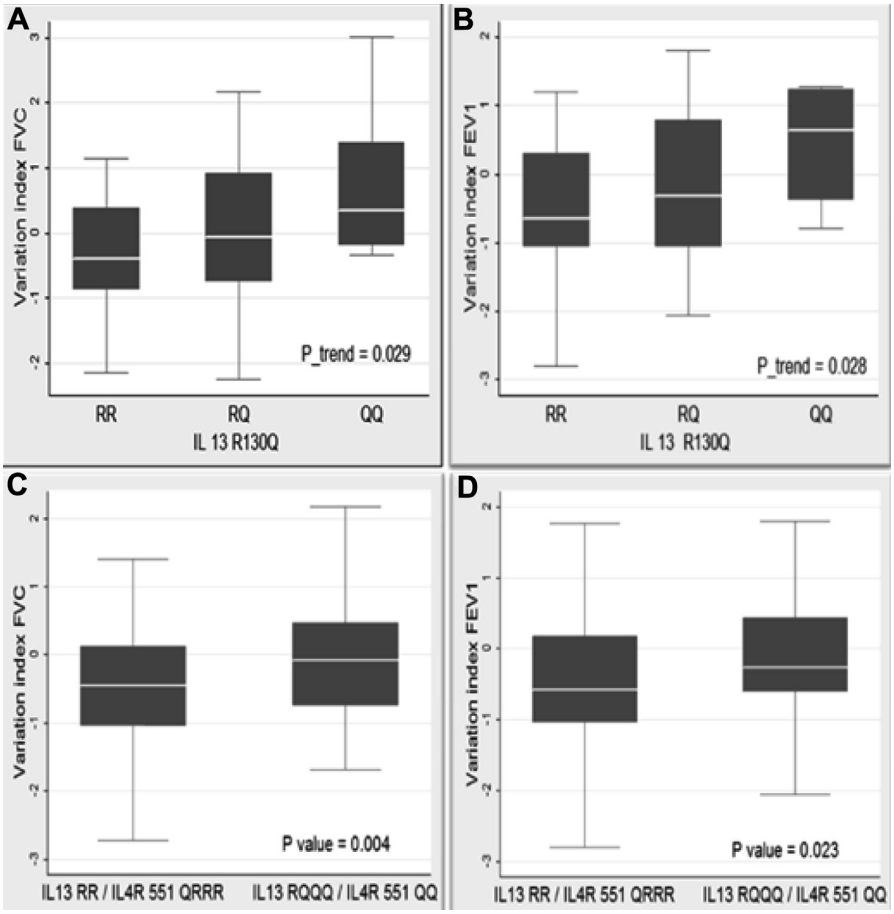
	Bakers and pastry makers with BHR		Hairdressers with BHR		*P* value[a]	All apprentices with BHR		*P* value[a]
	No.	% [95% CI]	No.	% [95% CI]		No.	% [95% CI]	
TNFA -308 (rs1800629)								
GG	159	74.0 [68.0-80.0]	113	75.8 [69.0-82.8]	.74	51	83.6 [72.3-90.8]	.24
GA	47	21.9 [16.3-27.4]	32	21.5 [14.8-28.1]		9	14.6 [8.02-25.78]	
AA	9	04.2 [01.5-06.9]	4	02.6 [0.7-05.2]		1	01.6 [0.4-08.7]	
Allele G	365	84.9 [81.2-88.0]	258	86.6 [82.0-89.0]	.52	111	91.0 [84.7-94.9]	.06
Allele A	65	15.1 [12.0-18.8]	40	09.3 [06.9-12.4]		11	09.0 [05.1-15.4]	
IL1A -889 (rs1800587)								
CC	97	45.1 [38.4-51.8]	71	50.4 [42.0-56.7]	.32	30	51.7 [39.1-64.1]	.35
CT	95	44.2 [37.5-50.9]	61	43.2 [35.0-51.5]		21	36.2 [25.0-49.1]	
TT	23	10.7 [06.5-14.9]	9	06.4 [02.3-10.4]		7	12.1 [06.0-22.3]	
Allele C	289	67.2 [62.5-71.3]	203	71.7 [66.5-76.9]	.18	81	69.8 [60.9-77.4]	.85
Allele T	141	32.8 [28.5-37.4]	79	28.0 [23.1-33.5]		35	30.2 [22.6-39.1]	

Abbreviations: CI, confidence interval; BHR, bronchial hyperresponsiveness.
[a]Fisher exact test for categorical variables, with Bonferroni correction for multiple testing.

eTable 5
Association of gene variants and significant gene-gene combination of variants with the variation of the FEV_1/FVC ratio and FeNO during the study period (univariate analysis)

Gene	Polymorphism	Genotype	FEV_1/FVC index,[a] mean ± SD		*P* value[b]	FeNO index,[a] mean ± SD		*P* value[b]
			With genotype	Without genotype		With genotype	Without genotype	
IL4RA S478P	rs1805015 (n = 333)	SS	$-3.16.10^{-5} \pm 1.35.10^{-4}$	$-3.47.10^{-5} \pm 1.49.10^{-4}$	.87	$1.99.10^{-4} \pm 1.61.10^{-4}$	$-1.27.10^{-}4 \pm 1.97.10^{-3}$	.30
IL4RA Q551R	rs180275 (n = 338)	QQ	$-2.65.10^{-5} \pm 1.31.10^{-4}$	$-4.75.10^{-5} \pm 1.60.10^{-4}$	.22	$1.05.10^{-4} \pm 1.66.10^{-4}$	$1.25.10^{-}4 \pm 2.39.10^{-3}$	.94
IL13 R130Q	rs20541 (n = 284)	RR	$-3.33.10^{-5} \pm 1.45.10^{-4}$	$-2.52.10^{-4} \pm 1.34.10^{-4}$	.67	$4.96.10^{-5} \pm 1.58.10^{-4}$	$2.16.10^{-}4 \pm 3.11.10^{-3}$	.61
TNFA -308	rs1800629 (n = 364)	GG	$-3.33.10^{-5} \pm 1.45.10^{-4}$	$-2.77.10^{-5} \pm 1.24.10^{-4}$	.75	$1.61.10^{-4} \pm 1.23.10^{-4}$	$1.23.10^{-}4 \pm 3.17.10^{-3}$	.32
IL1A -889	rs1800587 (n = 356)	CC	$-3.11.10^{-5} \pm 1.24.10^{-4}$	$-3.02.10^{-5} \pm 1.54.10^{-4}$	.96	$-5.61.10^{-6} \pm 2.06.10^{-4}$	$2.73.10^{-}4 \pm 2.08.10^{-3}$	.28
IL5 -703	rs2069821 (n = 371)	CC	$-2.52.10^{-5} \pm 1.32.10^{-4}$	$-3.86.10^{-5} \pm 1.45.10^{-4}$	.38	$1.60.10^{-4} \pm 1.86.10^{-4}$	$2.42.10^{-}5 \pm 2.26.10^{-3}$	.59
IL13 RQQQ/*IL4RA* 478 SS	rs20541/rs1805015	RQQQ/SS	$-2.94.10^{-5} \pm 1.24.10^{-4}$	$3.24.10^{-5} \pm 1.48.10^{-4}$	.93	$2.96.10^{-4} \pm 3.32.10^{-3}$	$2.28.10^{-5} \pm 2.11.10^{-3}$	.58
IL13 RQQQ/*IL4RA* 551 QQ	rs20541/rs180275	RQQQ/QQ	$-3.42.10^{-5} \pm 1.29.10^{-4}$	$-2.99.10^{-5} \pm 1.45.10^{-4}$	.77	$-3.77.10^{-5} \pm 2.91.10^{-3}$	$1.41.10^{-4} \pm 2.35.10^{-3}$	.98
TNFA GA/*IL5* TT	rs1800629/rs2069821	GA/TT	$1.67.10^{-5} \pm 8.78.10^{-5}$	$-3.52.10^{-5} \pm 1.44.10^{-4}$	.04	$-4.39.10^{-5} \pm 3.15.10^{-3}$	$8.41.10^{-5} \pm 2.19.10^{-3}$	.76

Abbreviations: FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity.
[a]The evolution index of parameters of airway inflammation measures the variation of each parameter between the inclusion visit and the last determination using the formula (last determination − determination at enrollment) vs (follow-up duration).
[b]One-way analysis of variance.



eFigure 1. Variation of forced vital capacity (FVC) (A) and forced expiratory volume in 1 second (FEV_1) (B) among nonatopic bakers and pastry maker apprentices according to *IL13* R130Q shows that RR genotype predicted a lower decrease of FVC (A) and FEV_1 compared with $_{RQ\ and\ QQ}$ genotypes. The association between the *IL13R* 130Q and *IL4RA* Q551R and the variation index of FVC (C) and FEV_1 (D) among nonatopic apprentices shows that the combination *Il13* RR with *Il4R* 551 QRRR was a significant predictor of a lower decrease of FVC and FEV_1.

eReferences

[1] Hilding AC. Simple method for collecting near-normal human nasal secretion. *Ann Otol Rhinol Laryngol*. 1972;81:422–423.
[2] Gueant-Rodriguez RM, Gueant JL, Viola M, Tramoy D, Gaeta F, Romano A. Association of tumor necrosis factor-alpha -308g>a polymorphism with IgE-mediated allergy to betalactams in an Italian population. *Pharmacogenomics J*. 2008;8:162–168.
[3] Bosco P, Gueant-Rodriguez RM, Anello G, et al. Association of il-1 rn*2 allele and methionine synthase 2756 aa genotype with dementia severity of sporadic Alzheimer's disease. *J Neurol Neurosurg Psychiatry*. 2004;75:1036–1038.
[4] Mounier-Geyssant E, Oury V, Mouchot L, Paris C, Zmirou-Navier D. Exposure of hairdressing apprentices to airborne hazardous substances. *Environ Health*. 2006;7:23.
[5] Mounier-Geyssant E, Barthélemy JF, Mouchot L, Paris C, Zmirou-Navier D. Exposure of bakery and pastry apprentices to airborne flour dust using PM2.5 and PM10 personal samplers. *BMC Public Health*. 2007;7:311.