ERJ Express. Published on April 11, 2007 as doi: 10.1183/09031936.00123206

B_2 adrenoceptor polymorphisms and asthma phenotypes: interactions with passive smoking

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Running head: *B_2AR* and passive smoking

Abstract

The aim of the study is to assess the possible interactions between (*B_2AR*) gene polymorphisms and passive smoking on forced expiratory volume in 1 second (FEV1), forced expiratory vital capacity (FVC) and exhaled nitric oxide (eNO) in children at age 11.

A cross-sectional analysis of the longitudinal cohort was conducted for associations between (*B_2AR*) gene polymorphisms and lung function and eNO with regard to passive smoking.

In children exposed to tobacco smoke, those with Arg16 (at least one Arg allele) had lower adjusted means of FEV1 (2.19 vs 2.38L; p=0.006) and FVC (2.43 vs 2.64L; p=0.011) compared with Gly16 homozygotes. Those with Gln27 (at least one Gln allele) also had a lower adjusted mean of FEV1 relative to Glu27 homozygotes (2.24L vs 2.39L; p=0.048). In children with no exposure to smoking those with Arg16 or Gln27 had lower adjusted geometric means of exhaled nitric oxide (eNO) compared with Gly16 homozygotes (15.4 vs.30.9 ppb; p=0.01) and Glu27 homozygotes (18.0 vs. 49.7 ppb; p=0.001), respectively.

In conclusion, passive smoking had a significant effect on associations between B_2 adrenoceptor polymorphisms and asthma-related phenotypes, enhancing the relationship between Arg16 and lung function and removing the relationship between Arg16 or Gln27 and eNO.

Key words: asthma, exhaled nitrogen oxide; genetics; lung function; passive smoking

Introduction

The B_2-adrenoceptor (*B_2AR*) is encoded by an intronless gene, located on chromosome 5q31-32 [1], and contains several reported single nucleotide polymorphisms (SNPs) [2]. The most commonly cited SNPs occur at nucleotide positions 46(G/A) and 79(C/G) and result in the substitution of glycine for arginine at codon 16 and glutamic acid for glutamine at codon 27, respectively. [3]. Although the *B_2AR* is not considered to be a major susceptibility gene for asthma [4], *B_2AR* variant alleles have been suggested to play a role in intermediate or asthma-associated phenotypes, such as response to medications [5, 6], airway hyperresponsiveness [7] and asthma severity [8]. However, studies on the association between asthma-related phenotypes and the B_2-adrenoceptor polymorphisms have reported inconsistent results [9]. Environmental factors may interact with the *B_2AR* polymorphisms to affect asthma-associated phenotypes [7]or even asthma susceptibility [10]. In terms of interactions between *B_2AR* and smoking, there is evidence that metabolites of the tobacco-specific nitrosamine stimulate the growth of human adenocarcinoma cells and are high affinity agonists for *B*-adrenergic receptors [11, 12]. Moreover, a genome screen study of inherited risk for asthma and bronchial responsiveness reported that the influence of susceptibility genes for asthma might not be apparent without the appropriate exposure to common environmental stimuli, such as passive smoking [13]. Passive smoking exposure has been suggested to be a risk factor for the development of asthma [14]. Since children are commonly exposed to cigarette smoke at home, in automobiles and public places, the interaction between cigarette smoke exposure and *B_2AR* polymorphisms is important for a better understanding of the association between these genetic variants and asthma-associated phenotypes.

However, only a few studies [7, 10, 15] have investigated the interaction between cigarette smoking and *B_2AR* polymorphisms and none have examined children exposed to passive smoke.

An ongoing longitudinal study in Perth has measured lung function and airway responsiveness in a cohort from the age of one month up to 11 years [16, 17] and provided the opportunity to study the interactions between *B_2AR* polymorphisms and passive smoking on asthma-associated phenotypes. We have previously described the associations between Arg16 and decreased forced expiratory volume in 1second (FEV1) at age 11 in this cohort [17]. We have also reported the complex relationships between eNO, atopy and airway responsiveness for this cohort [18]. The current study analysed whether passive exposure to cigarette smoke might influence the association of *B_2AR* polymorphisms with both lung function and eNO. This is the first investigation of possible interactions between *B_2AR* polymorphisms and passive smoking for lung function and levels of eNO.

Methods

Subjects

Initially, 253 Western Australian children were randomly recruited from an unselected, Caucasian population before birth during the years 1987 to 1991 [19]. They were all of European origin, born at full term, none had major congenital anomalies and infants were first assessed at approximately 1 month of age [16]. At age 11, 194 children were followed up with 180 children genotyped for Arg16Gly and Gln27Glu. Among them, 168 children had lung function measured and in 86 children exhaled nitric oxide levels were determined. The interaction between *B_2AR*

polymorphisms and passive smoking was examined for the outcomes of lung function and exhaled NO levels in this population.

Lung function, exhaled NO and other asthma associated phenotypes

Pulmonary function testing was performed using a spirometer (Pneumocheck Spirometer 6100) in accordance with published guidelines [20]. Exhaled nitric oxide was measured using a fast response chemiluminescence analyser, as previously described [18, 21]. Airway responsiveness to histamine was evaluated using the rapid dosimeter technique [18, 22] and the dose response slope (DRS) was calculated. Skin prick allergy tests were conducted for cow's milk, egg white, rye grass, mixed grass, *Dermatophagoides farinae, D. pteronyssinus,* cat dander, dog dander, *Alternaria alternans* and *Aspergillus fumigatus* as described by Pepys [23]. Atopy was defined as the presence of at least one positive skin prick test (the longest dimension of weal ≥3mm). The eosinophil count was measured using a flow cytometer (Coulter Maxm, Beckman-Coulter Inc).

Genotyping for B_2AR polymorphism

Blood was collected for DNA extraction when reviewed at either 6 or 11 years of age. SNPs at codons 16 and 27 were genotyped using techniques described in detail previously [17, 24]. Haplotypes were constructed using the Bayesian statistical based program PHASE, version 2.0.2 [25].

Passive smoking assessment

In the study cohort, passive exposure to cigarette smoke was investigated on three occasions: at enrolment and follow up at both ages 6 and 11 years. Specific questions

related to maternal and paternal smoking in pregnancy were asked at enrolment. Information on current smoking, smoking history and smoking habit for parents and other smokers in households was also collected at enrolment and during follow up at both ages 6 and 11 years. Parents were asked the following questions with respect to cigarette smoke exposure:

Have you ever smoked cigarettes?

Are you a current smoker?

Do you now smoke cigarettes?

How old were you when you started smoking regularly?

If you stopped smoking completely, how old were you?

In addition to parents, were there any other smokers at home?

If a current smoker, when you are at home where do you smoke?

Based on the information on passive exposure to cigarette smoke collected on the three occasions, the children were divided into three groups: "no exposure", "significant exposure" and "unknown exposure". "No exposure" was defined as the child never having lived with a current smoker and his/her parents did not smoke in pregnancy. "Significant exposure" was defined as the child having lived with at least one smoker for more than one year during infancy and childhood (up to 11 yrs of age) and whose parents possibly smoked in pregnancy. The other children were defined as "unknown exposure".

Statistical analysis

Both Forced Expired Volume in one second (FEV1) and Forced Vital Capacity (FVC) had approximately normal distributions and parametric analysis was employed

directly. Levels of eNO and the confounders, DRS and eosinophil counts, were $\log_e$ transformed prior to parametric analysis. ANOVA was employed to compare the differences in FEV1, FVC and eNO levels between genotypes of Arg16Gly and Gln27Glu in a univariate analysis. General linear models , adjusting for the confounders, were conducted to compare the adjusted means/geometric means of FEV1 and FVC and eNO levels both between Arg16 carriers (at least one Arg allele) and Gly16 homozygotes and between Gln27 carriers (at least one Gln allele) and Glu27 homozygotes. Age, gender and height were selected as confounders for lung function parameters and age, gender, height, atopy, DRS and eosinophil counts had been adjusted. Due to the sample size, we only investigated the dominant effects of Arg in Arg16Gly and Gln in Gln27Glu in the current study. These models were fitted in the whole population, as well as in children with no exposure to tobacco smoke and in children with significant exposure to smoke separately. Adjusted means (or geometric means) and the corresponding standard errors (SEs) (or upper 95% confidence intervals) are presented in Figures 1,2 and 3. Regarding the associations of haplotypes of *B_2AR* with FEV1, FVC and eNO, linear regressions were employed after adjusting for the confounders of interest. Statistical analysis was performed using SPSS v11.5 for Windows except for using STATA (Intercooled Stata 9) to estimate the adjusted means. The significance was assumed at the 5% level.

Results

Details of the characteristics in the population

The study population included 180 children successfully genotyped for *B_2AR* polymorphisms and followed up at age 11 years. The characteristics of the population are shown in table 1. In summary, the mean age of the population was 10.9 yrs (95%

CI: 10.8-11.1), with 77/180 females (42.8%). With regard to *B_2AR*, 61.7 % of the population had at least one Arg allele at codon 16 and 78.4% had at least one Gln allele at codon 27. There was significant linkage disequilibrium between the *B_2AR* polymorphisms ($p<0.001$). Approximately 46.1% of the population was classified as having "no exposure" to tobacco smoke; and 37.2% as having "significant exposure". Eighty-nine of the 171 children (52%) were atopic as defined by skin-prick test at age 11. Lung function was done on168 children and showed mean FEV1 and FVC of

Table 1

2.29L (95% CI: 2.23-2.36L) and 2.51L (95% CI: 2.43-2.58L), respectively. Exhaled nitric oxide was determined in 86 children and the geometric mean (GM) was 11.0 ppb (95% CI: 9.1-13.2ppb).

Lung function and B_2AR polymorphisms

Table 2 shows the association between lung function parameters and *B_2AR* polymorphisms. In unadjusted univariate analysis, no significant differences in FEV 1 and FVC were found between both Arg 16 (at least one Arg allele) and Gly 16 homozygotes and between Gln 27 (at least one Gln allele) and Glu 27 homozygotes in the 168 children. After adjusting for age, gender and height, Arg 16 was associated with significantly decreased FEV1 ($p=0.013$) and FVC ($p=0.012$), compared with Gly 16 homozygotes. For the % predicted values of lung function, Arg 16 was also associated with significantly decreased %FEV1 (104 vs. 108; $p=0.027$) and %FVC (100 vs. 104; $p=0.018$), compared with Gly 16 homozygotes. The influence of Gln27Glu on lung function was still not statistically significant after this adjustment.

Table 2

The relationship between *B_2AR* polymorphisms and lung function parameters (FEV1 and FVC) in children with "no exposure" and "significant exposure" to tobacco

smoke were analysed separately using general linear models. For the Arg16Gly polymorphism, Arg16 was associated with significantly lower adjusted means of FEV1 and FVC (2.19 vs 2.38L; p=0.006 and 2.43 vs 2.64; p=0.01 respectively) in children with "significant exposure" to tobacco smoke, compared with the Gly16 homozygotes (See Figure 1). Children with Gln27 had a significantly lower mean FEV1 relative to Glu27 homozygotes (2.24 vs 2.39L; p=0.048) in those with "significant exposure" to tobacco smoke (See Figure 2). In children with "no exposure" to tobacco smoke, the association between lung function parameters and both Arg16 and Gln27 was not statistically significant.

Figure 1

Figure 2

Exhaled nitric oxide and B_2AR polymorphisms

Eighty-six children genotyped for *B_2AR* polymorphisms also had eNO levels measured. The concentrations of eNO in the different *B_2AR* genotype groups are shown in Table 3. Significant differences were found in levels of eNO between the *B_2AR* Arg16Gly genotypes in both the whole population (86 children) (p=0.017) and the 38 children (p=0.012) with "no exposure" to tobacco smoke.

Table 3

Children with Arg16 appeared to have lower levels of eNO, compared with Gly16 homozygotes. The mean increase of eNO levels in children with "significant exposure" to tobacco smoke was not significant. Children with Gln27 had lower levels of eNO, which was not significant.

In order to adjust for covariates, general linear models were fitted for eNO levels (the dependent variable) with either Arg16 or Gln27 as independent variables. The confounders included in the models for the adjustment were age, gender, height,

atopy, DRS and eosinophil counts. These confounding variables have previously been found to be significantly associated with levels of eNO in children [18]. After the adjustment, children with Arg16 still had a significantly lower level of eNO, compared to Gly16 homozygotes (p=0.001). Furthermore, children with Gln27 also had significantly lower concentrations of eNO, relative to Glu27 homozygotes (p=0.018).

Figure 3

Figure 3 shows the adjusted geometric means of eNO by the different genotypes in children with "no exposure" and "significant exposure" to tobacco smoke. As found in the whole population, Arg16 was associated with significantly lower levels of eNO (15.4 vs. 30.9 ppb; p=0.01) in children with "no exposure" to smoke compared with Gly16 homozygotes. Gln27 was significantly associated with decreased levels of eNO in children with "no exposure" to smoke, compared with the Glu homozygotes (18.0 vs 49.7 ppb; p=0.001). However, the decrease in eNO levels for Arg16 and Gln27 were not statistically significant in children with "Significant exposure" to tobacco smoke.

Haplotypes of B_2AR polymorphisms

Four haplotypes were constructed for the two SNPs of *B_2AR,* namely ARGGLU*,* ARGGLN, GLYGLU and GLYGLN. Due to the linkage disequilibrium between the two SNPs, ARGGLU was rare, therefore, only the other three haplotypes were further investigated for the association with lung function and eNO, stratified by passive smoking exposure. The frequencies of ARGGLN, GLYGLU and GLYGLN in the population were 37.5%, 41.7% and 17.5%, respectively.

Table 4

Table 4 shows the results for the associations of haplotypes of *B_2AR* with FEV1, FVC and eNO in children with and without exposure to passive smoking. In children with "No exposure", those with the GLYGLN haplotype had significantly higher FEV1 and FVC with p values of 0.009 and 0.002, respectively. Moreover, those with the ARGGLN haplotype had lower eNO (p=0.01) and those with the GLYGLU haplotype higher eNO (p=0.04). In children with "Significant exposure", those with the ARGGLN haplotype had significantly lower FEV1 (p=0.011) and FVC (p=0.021). Those with the GLYGLU haplotype had relatively higher FEV1 (p=0.024). Acknowledging the small sample size in children with the GLYGLN haplotype, the relationships between the haplotype GLYGLN and both lung function parameters and eNO were re-examined in linear regressions by grouping children with either one or two copies of GLYGLN together. The GLYGLN haplotype was still significantly associated with FEV1 and FVC in children with "no exposure".

Discussion

This study showed significant effects of passive smoking on associations of *B_2AR* gene polymorphisms with lung function and eNO. In children with exposure to tobacco smoke during pregnancy, infancy and childhood, Arg16 and Gln27 were associated with a decrease in the forced expiratory volume in 1s and forced expiratory vital capacity (not statistically significant for Gln27). In children with "no exposure", the effects of Arg16 and Gln27 on lung function were not significant. Arg16 and Gln27 carriers had significantly decreased levels of eNO, with the association more apparent in children with "no exposure" to tobacco smoke, suggesting that smoke exposure removed the difference between alleles. The haplotype analyses further confirmed the modifying effects of passive smoking on the associations.

Previous studies have found that the B_2-adrenoceptor gene polymorphisms are of functional importance [2]. We have previously reported the relationship between Arg16 and increased bronchial hyperresponsiveness at 1 month of age and decreased lung function at 11 years of age [17]. We have also investigated the associations between bronchial hyperresponsiveness and lung function and haplotypes of *B_2AR* gene [26]. The present study clarified the association of *B_2AR* gene and lung function and eNO, particularly in children with exposure to the environmental factor of passive smoking.

There have been two epidemiological studies reporting the interaction between B_2AR polymorphisms and smoking. The characteristics and findings of the two studies plus the present study are summarised in Table 5. There are significant differences in terms of the characteristics of the three populations in that the current study involved passive smoking in children, while the other two analysed active smoking in adults. The ethnic background of the cohorts and observed outcomes also differ between the three studies. Wang *et al*. [10] found that there was a significant dose response relationship between tobacco smoke (pack-year) and risk (odds ratio) of asthma in Arg16 homozygotes in a middle-aged Chinese population. In that study, lower predicted FEV1 was one of the important discriminating criteria between asthmatic and control subjects. The findings in our study are consistent with Wang's report in that there was an interaction between Arg16 and smoking on lung function. The marginally significant finding that Gln27 was associated with decreased FEV1 in children with significant exposure to smoking in our study may possibly be due to linkage disequilibrium between the Arg16Gly and Gln27Glu SNPs. Litonjua *et al*.[7]

did not find an association between Arg16 and airway responsiveness in ever-smokers in a relatively older white male population. They attributed their contradictory findings to the differences in ethnicity, gender and asthma phenotypes when their cohort was compared to Wang's study. Our study also found that Arg16 and Gln27 were associated with significantly decreased levels of eNO. Although the phenotypes and smoking exposure type were different from those reported by Litojua [7], the results are consistent in that the association was more apparent with no exposure to smoke. Litojua *et al*. [7]suggested that the stronger association in non-smokers resulted from smoke exposure overwhelming any effects of B_2AR gene variants in ever-smokers. However, this is unlikely to be the explanation in our study as no significant difference was found in levels of eNO between children with "significant exposure" and "no exposure" to smoking. Wang *et al*.[10] reviewed the effects of tobacco smoke on the density of B_2AR, as reported by Laustiola *et al*. [27, 28], and speculated that the smoking-induced downregulation of the B_2AR might differ in subjects carrying different polymorphic forms of this receptor [10]. They suggested that this spectrum of gene smoking-exposure interactions might contribute to variable expression of the asthma related phenotypes [10]. The complex interactions between B_2AR polymorphisms, passive smoking, lung function, eNO and asthma susceptibility require further elucidation.

Exhaled nitric oxide has been shown to be raised in atopy [18, 29, 30]. Although not consistently, exposure to environmental tobacco smoke appeared to decrease eNO [31-34]. In the current study, the unique finding of the association between B_2AR polymorphisms and eNO levels was independent of several asthma associated phenotypes. Increased levels of nitric oxide are the result of enhanced expression of

nitric oxide synthases in various cells in the lung including alveolar macrophages, airway smooth muscle and airway epithelial cells [35]. No significant evidence supports a direct association between *B_2AR* and nitric oxide synthases, however, there are several possible indirect links that have been suggested. Firstly, nitric oxide synthases may be transcriptionally regulated in response to cytokines [36, 37] while cytokines may be regulated or inhibited by *B_2AR* [38, 39]. Secondly, nitric oxide, as a messenger molecule, is related to endothelial mechanisms for vasodilation due to the stimulation of *B_2AR* [40]. It has been suggested that N^G-monomethyl-LARginine (L-NMMA) inhibits the response to *B* -agonists by activation of the endothelial LARginine/NO pathway [40]. These possible relationships between *B_2AR* and eNO may partly explain the variations of eNO associated with different *B_2AR* polymorphisms. Recognising the possible effects of passive smoking on eNO, it is possible that exposure to passive smoking may modify the effects of *B_2AR* polymorphisms on eNO.

There are certain limitations to the present study. Genotyping was only done in 180 of the 253 children recruited at the beginning of the study. Among the 180, some did not have lung function tests or measurements of eNO at age 11 years. In addition, boys were dominant in the population. Analysis of only those subjects with complete information on genotyping, lung function or eNO may have introduced bias to the findings. Missing data and gender disproportions may result in the population not being representative in general. Stratification by passive smoking further decreased the sample size for the linear regression analysis conducted in each group. Therefore, the findings should be interpreted with caution. However, no significant differences were found in terms of asthma, atopy and other asthmatic symptoms between the

population studied and those without genotyping or lung function or eNO measurements. This suggested that the study population is likely to represent the whole cohort. Although passive smoking exposure was assessed several times on questionnaire surveys, no biomarkers of smoking exposure were measured due to financial and other constraints. Possible parent recall biases may thus have distorted the findings. However, as recently reported, questionnaire assessment reliably discriminated between different levels of passive smoking exposure in children [41]. In addition, an "Unknown exposure" group was defined to circumvent the possible uncertainty of smoking exposure in children. Those children with "unknown exposure" to cigarette smoke were not included in the analysis for the interaction between *B_2AR* polymorphisms and passive smoking.

Conclusion

We have shown that the *B_2AR* gene polymorphisms were associated with decreased lung function in children significantly exposed to cigarette smoke and with decreased eNO in children not exposed. There was a significant interaction between the Arg16Gly and Gln27Glu *B_2AR* SNPs and passive smoking for lung function and eNO in the population.

Acknowledgments

The authors are grateful to many colleagues who have contributed to the cohort study and we also acknowledge the important contribution of Dr Sally Young who recruited the cohort and of Drs Veena Judge, Peter Rye and Lyle Palmer who did the assessment of the cohort at age 6 years.

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Table 1 Characteristics of the population

	N	%
Gender (n=180)		
Girls	77	42.8
Arg16Gly (n=180)		
ArgArg	36	20.0
ArgGly	75	41.7
GlyGly	69	38.3
Gln27Glu (n=180)		
GlnGln	57	31.7
GluGln	84	46.7
GluGlu	39	21.7
Passive smoking (n=180)		
No exposure	83	46.1
Significant exposure	67	37.2
Unknown exposure	30	16.7
Atopy (n=171)		
Atopic	89	52.0

	n	Mean/ GM	95%CI Lower	95%CI Upper
Age (yrs)	180	10.9	10.8	11.1
Height (cm)	170	145	144	147
Eosinophils (cells/μL)	160	288	256	325
DRS (%)*	165	1.95	1.56	2.39
FEV1 (L)	168	2.29	2.23	2.36
FVC (L)	168	2.51	2.43	2.58
Exhaled NO (ppb)	86	11.0	9.1	13.2

DRS: Calculated as the percentage of reduction in FEV1 per micromole of histamine inhaled.

Table 2 Lung function and B2AR polymorphisms

	FEV1		FVC	
	N	Means (l)	N	Means (l)
Unadjusted*				
at least one Arg allele	103	2.26	103	2.47
Gly 16 homozygotes	65	2.34	65	2.57
p		0.25		0.21
at least one Gln allele	131	2.29	131	2.50
Glu 27 homozygotes	37	2.31	37	2.53
p		0.78		0.72
Adjusted@				
at least one Arg allele	103	2.25	103	2.46
Gly 16 homozygotes	65	2.35	65	2.58
p		**0.013**		**0.012**
at least one Gln allele	131	2.29	131	2.50
Glu 27 homozygotes	37	2.30	37	2.51
p		0.81		0.76

***:** Independent Sample T test; @: Univariate Analysis of Variance; the adjusted means were estimated after adjusting for age, gender and height.

Table 3 the geometric means and 95% CI of exhaled nitric oxide

		N	GM	95% CI		P*
				Lower	Upper	
86 children						
Arg16Gly	ArgArg	15	9.6	6.8	13.4	**0.017**
	ArgGly	37	8.6	6.3	11.7	
	GlyGly	34	15.2	11.3	20.5	
Gln27Glu	GlnGln	20	8.8	5.9	12.9	0.071
	GlnGlu	46	10.3	8.0	13.2	
	GluGlu	20	16.0	10.3	24.7	
38 children with "No exposure"						
Arg16Gly	ArgArg	3	6.9	1.9	25.2	**0.012**
	ArgGly	22	8.8	6.0	12.7	
	GlyGly	13	21.4	12.2	37.6	
Gln27Glu	GlnGln	7	9.5	2.9	31.1	0.097
	GlnGlu	24	10.1	7.3	14.1	
	GluGlu	7	23.4	9.7	56.2	
35 children with "Significant exposure"						
Arg16Gly	ArgArg	11	11.1	7.5	16.6	0.42
	ArgGly	9	9.9	5.2	19.1	
	GlyGly	15	14.6	9.7	21.8	
Gln27Glu	GlnGln	11	8.8	6.2	12.4	0.12
	GlnGlu	14	12.3	8.0	19.0	
	GluGlu	10	16.9	9.6	29.7	

*: ANOVA was conducted.

Table 4 Associations of haplotypes with FEV1, FVC and eNO*

		FEV1		FVC			eNO	
	n^1	Coefficients	p	Coefficients	p	n^2	Coefficients	p
No exposure								
ARGGLN	63	-0.03	0.55	-0.06	0.23	24	-0.55	**0.010**
GLYGLU	63	-0.05	0.24	-0.06	0.25	36	0.39	**0.040**
GLYGLN	31	0.14	**0.009**	0.18	**0.002**	12	-0.12	0.62
Significant exposure								
ARGGLN	48	-0.11	**0.011**	-0.12	**0.021**	26	-0.16	0.30
GLYGLU	58	0.10	**0.024**	0.07	0.15	29	0.25	0.058
GLYGLN	19	0.03	0.72	0.09	0.27	10	-0.35	0.16

*: Linear regressions were employed for the analysis

n^1: Number of the haplotypes with lung function determined in children with "no exposure" (80 children) and with "significant exposure" (67 children)

n^2: Number of the haplotypes with eNO determined in children with "no exposure" (38 children) and with "significant exposure" (35 children)

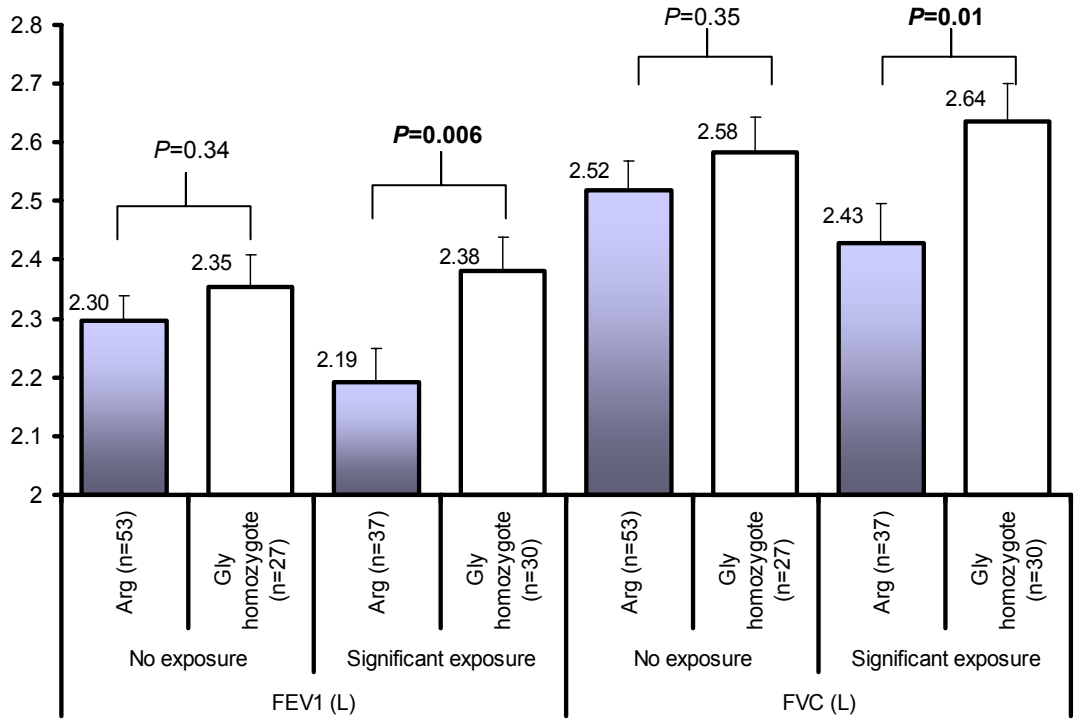

Figure 1 Adjusted means and standard errors of FEV1 and FVC in children with "no" or "significant exposure" to tobacco smoke between Arg16 (at least one Arg allele) and Gly16 homozygotes; General linear models were fitted to compare the difference in the adjusted means.

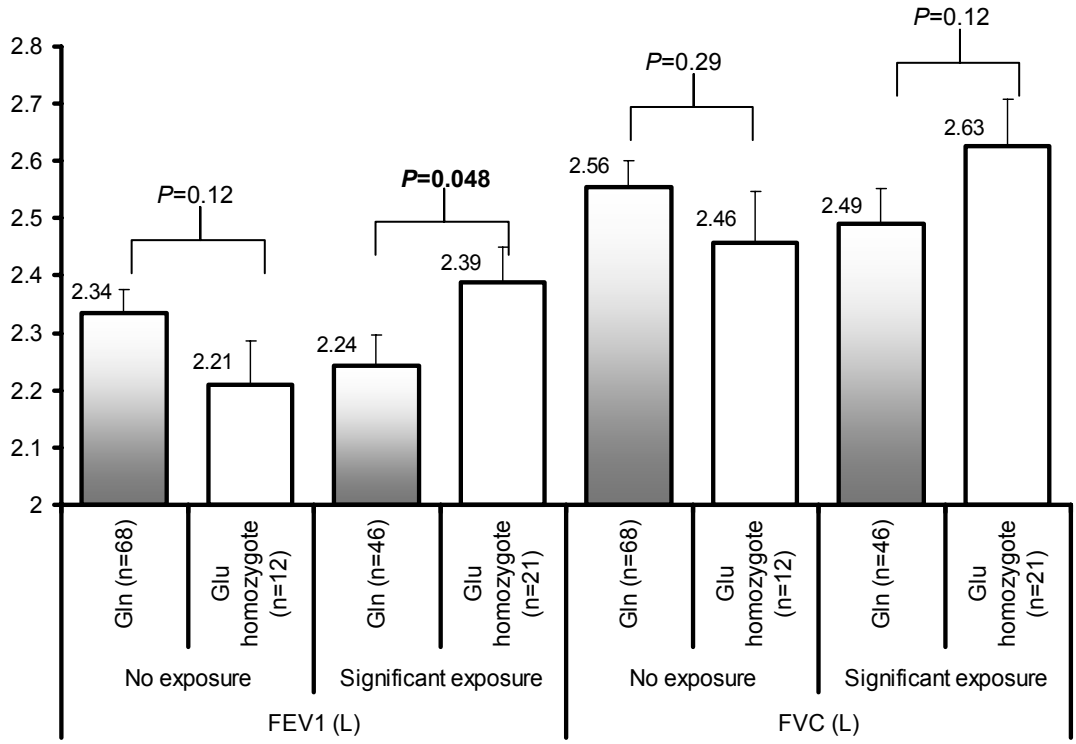

Figure 2 Adjusted means and standard errors of FEV1 and FVC in children with "no" or "significant exposure" to tobacco smoke between Gln27 (at least one Gln allele) and Glu27 homozygotes; General linear models were fitted to compare the difference in the adjusted means.

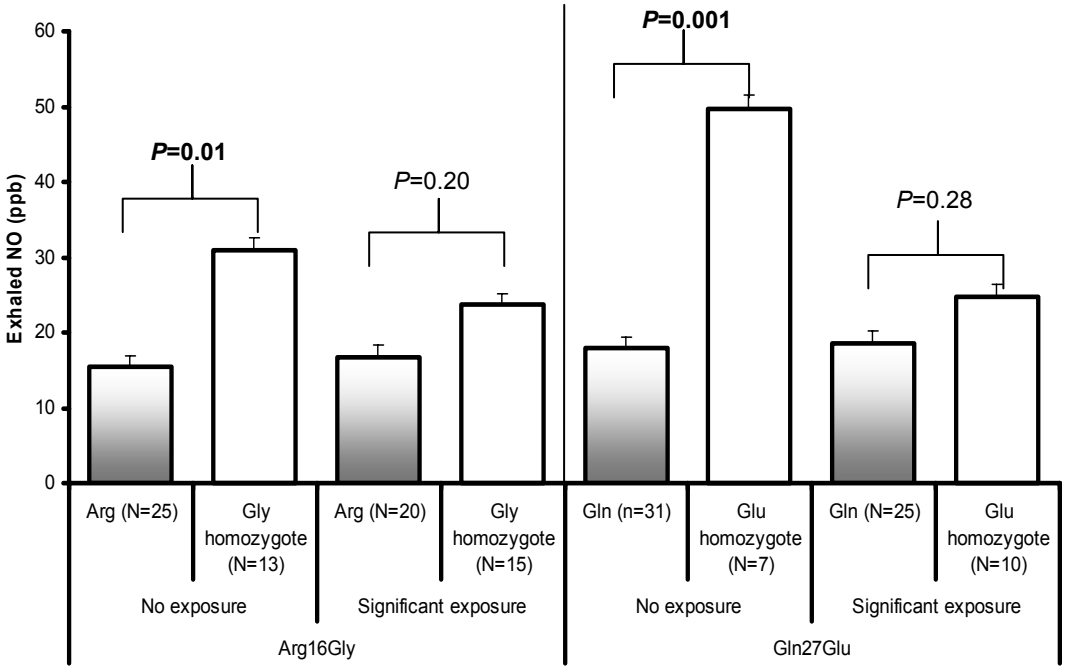

Figure 3 Adjusted geometric means and upper 95% CIs of exhaled nitric oxide (NO); Comparisons between Arg 16 (at least one Arg allele) and Gly16 homozygotes and between Gln27 (at least one Gln allele) and Glu27 homozygotes; General linear models were fitted to compare the difference in the adjusted geometric means.

Table 5 Characteristics and findings in the three studies on interactions between *B*2AR polymorphisms and smoking

	Population	Means of age	Outcomes observed	Type of smoking	Findings in subjects exposed to smoke	Findings in subjects not exposed to smoke
Zhang *et al.*	Children in Australia	10.9yrs	FEV1 FVC Exhaled NO	Passive	1. Arg16 was associated with decreased FEV1 and FVC; 2. Gln27 was associated with decreased FEV1	1. Arg16 was associated with decreased exhaled NO; 2. Gln27 was associated with decreased exhaled NO.
Litonjua *et al.[7]*	European-American	Cases: 63.1yrs Control subjects: 59.2yrs	AHR*	Active		1. Arg16 was associated with the increased risk of AHR 2. Gly16/Gln27 haplotype was associated with the decreased risk of AHR
Wang *et al.[10]*	Chinese in China	Cases: 30.6yrs Control subjects: 35.3	Asthma	Active	There was a dose response relationship between tobacco smoke and the risk of asthma in Chinese adults with Arg16 homozygote	

AHR: Airway hyperresponsiveness