

## Original article

 $\beta_2$ -ADR haplotypes/polymorphisms associate with bronchodilator response and total IgE in grass allergy

Association and linkage studies of  $\beta_2$ -adrenergic receptor ( $\beta_2$ -ADR) polymorphisms in relation to the expression of asthmatic phenotypes and immune regulatory mechanisms have shown inconsistent results. In order to analyse the relevance of particular combinations of single nucleotide polymorphisms (SNPs) or haplotypes of  $\beta_2$ -ADR gene to bronchial asthma, bronchodilator response and total immunoglobulin E (IgE) we determined by direct DNA sequencing five SNPs (in positions: -47, -20, 46, 79, 252) in a group of 180 Caucasian subjects (110 patients with grass allergy and 70 nonatopic controls). The eight different  $\beta_2$ -ADR haplotypes were identified, with three the most common of them representing 92% of the studied cohort. Significantly higher ( $p_{\text{cor}} = 0.0045$ ) bronchodilator response was observed in patients with homozygotic genotype 46A/A in comparison with respective homo- and hetero-zygotes. There was no significant difference in bronchodilator response when  $\beta_2$ -ADR haplotypes were analysed. Significantly higher ( $p_{\text{cor}} = 0.0005$ ) total IgE levels were found in patients with  $\beta_2$ -ADR haplotype -47T/-20T/46A/79C/252G and homozygotic carriers of 46A ( $p_{\text{cor}} = 0.0015$ ) and 79C ( $p_{\text{cor}} = 0.003$ ) genotypes. No significant associations were found in regards to asthmatic phenotype and atopy. These results indicate that depending on phenotype studied, either an individual  $\beta_2$ -ADR SNP or  $\beta_2$ -ADR haplotype might affect disease manifestation.

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The  $\beta_2$ -adrenergic receptor ( $\beta_2$ -ADR) belongs to family of G protein-coupled receptors that mediates the action of endogenous catecholamines-like adrenaline, noradrenaline and exogenous administered agonists. More than 10 single nucleotide polymorphisms (SNPs) were identified in the gene in the human population (1, 2). The nonsynonymous SNPs at nucleotides 46 and 79 that lead to changes in amino acid residues at position 16 (16 Arg/Gly, arginine/glycine) and 27 (27 Gln/Glu, glutamine/glutamic acid) have been the most extensively studied and are common variants in the general population. *In vitro* studies in cell lines and primary cultures of human airway smooth muscle cells demonstrated that 16 Gly allele confers enhanced agonist-induced down-regulation whereas 27 Glu variant imparts resistance to down-regulation (3, 4). Another interesting SNP was identified at position -49 T/C that results in a change of the last amino acid of short peptide [termed  $\beta_2$ -ADR upstream peptide (BUP)], thus affecting  $\beta_2$ -ADR expression (5).

Several association and linkage studies related to polymorphisms of  $\beta_2$ -ADR were published showing inconsistent results. It has been shown that bronchodilator response to  $\beta$ -agonists or the extend of the process of receptor down-regulation correlates with an individual  $\beta_2$ -ADR SNPs (6–8), however, other studies failed to show any such relations (9, 10). Some data suggested that these

polymorphisms might be associated with asthmatic phenotype (11, 12), bronchial hyperresponsiveness (13, 14) and total serum immunoglobulin E (IgE) levels (6), although other studies showed discordant results (15–17).

This inconsistency may be partly explained by the fact that most of the above studies were designed to study relationships of single SNP in isolation (mainly SNPs at positions 46 and 79), not taking into account other  $\beta_2$ -ADR SNPs and the relevance of combinations of multiple SNPs or specific haplotypes. The results of recent study of Drysdale et al. (18) indicate that it is rather the unique interaction of multiple SNPs within a haplotype what determines functional relevance of particular polymorphisms at  $\beta_2$ -ADR locus. Studies with other polymorphic genes have also shown advantages of analysing haplotypes rather than single polymorphisms (19, 20). However, when extended haplotypes of  $\beta_2$ -ADR were studied in relation to cAMP response and  $\beta_2$ -ADR expression in peripheral blood mononuclear cells (PBMCs), no correlation was found (21). The other problem, not enough appreciated to date, this is a selection method of SNPs genotyping. Most population studies have used modified polymerase chain reaction (PCR)-based methods that might result in some percentage of false genotyping when compared with reference method such as DNA sequencing (22). Another cause of

such variable results may be related to problems with selection of appropriate, clinically and genetically homogenous populations (rarely comparable among studies) and the fact that genetic contribution of β<sub>2</sub>-ADR SNPs to studied disease phenotypes is rather weak.

We have therefore designed a study in a genetically homogenous, clinically well-defined population of patients with grass allergy to test association of five common β<sub>2</sub>-ADR SNPs at positions: –49 T/C; –20 T/C; 46 A/G; 79 C/G and 252 G/A, genotyped by DNA sequencing and combined into haplotypes, with asthmatic phenotype, bronchodilator response and total serum IgE levels. The degree of linkage disequilibrium occurring between these polymorphic loci has also been assessed.

## Methods

### Subjects

A 180 unrelated subjects living in the central area of Poland were included into a study. A group of 110 patients with grass pollen allergy manifesting as seasonal rhinitis and/or asthma (56 women, 54 men, mean age 27.15 years, range: 17–61) were randomly selected from patients treated in the Outpatient Unit of the authors' department. All subjects had a clear history of seasonal symptoms from May to July for at least three consecutive seasons. Based on personal history and clinical examination during grass pollen seasons the patients were classified into rhinitis only group (*N* = 56), and into asthma/rhinitis group (*N* = 54) if they had a history of asthma symptoms together with symptoms of rhinitis during at least two consecutive grass pollen seasons. None of the patients had received allergen immunotherapy. Allergy to grass pollen was confirmed by a positive skin prick tests to mixed grass pollens (Allergopharma, Reinbeck, Germany). Mean wheal diameter of skin prick test to mixed grass pollens and prevalence of positive skin prick tests to other allergens tested (*Dermatophagoides farinae*, *D. pteronyssinus*, *Alternaria* species, cat dander, dog dander, mixed tree pollens, mixed weed pollens) did not differ significantly between both groups (Table 1).

The control nonatopic group consisted of 70 unrelated subjects (ethnically similar to a case group) with negative history of atopic diseases, no history of allergy in parents or siblings and with negative skin prick tests to a panel of inhaled allergens.

Table 1. Characteristics of studied groups

|                                                                         | Rhinitis   | Asthma/rhinitis | Nonatopic controls |
|-------------------------------------------------------------------------|------------|-----------------|--------------------|
| <i>N</i>                                                                | 56         | 54              | 70                 |
| Mean age (year ± SD)                                                    | 26.9 ± 8.3 | 27.4 ± 9.5      | 27.5 ± 7.6         |
| Range (years)                                                           | 17–50      | 19–61           | 20–48              |
| Female/male                                                             | 28/28      | 28/26           | 38/32              |
| Active smokers (%)                                                      | 10.7       | 9.2             | 10.0               |
| Mean ΔFEV <sub>1</sub> (%) ± SD)                                        | 3.7 ± 3.0  | 10.7 ± 4.6      | –                  |
| FEV <sub>1</sub> /FVC (%) ± SD)                                         | 83.5 ± 7.2 | 81.6 ± 6.9      | –                  |
| Mean wheal diameter of skin prick test to mixed grass pollens (mm ± SD) | 11.5 ± 4.2 | 12.1 ± 4.7      | 0.0                |

FEV<sub>1</sub>, forced expiratory volume in 1 s; FVC, forced vital capacity.

The study was approved by Medical University of Lodz Ethical Committee.

### Bronchodilator response

Spirometry was performed according to American Thoracic Society criteria (23) by using ABCPneumo 2000RS spirometer (Abc-Med, Cracow, Poland) during pollen seasons in all patients studied. Patients were not taking inhaled steroids, long-acting β<sub>2</sub>-agonists and short-acting β<sub>2</sub>-agonists were withdrawn for at least 2 days. At least three measurements in the standing position were performed and the best result was recorded. Then 200 µg of salbutamol was administered by metered dose inhaler with a spacer followed by a second spirometry measurement after 15 min. Changes in forced expiratory volume in 1 s (ΔFEV<sub>1</sub>) before and after drug inhalation were calculated according to the following formula: 100 × (postsalbutamol FEV<sub>1</sub> – initial FEV<sub>1</sub>)/initial FEV<sub>1</sub>.

### Total serum IgE measurements

In all subjects total serum IgE levels during pollen season were determined using the enzyme-linked immunosorbant assay (ELISA) method (Sanofi Pasteur, Paris, France) according to manufacturer's protocol.

### β<sub>2</sub>-Adrenoceptor polymorphism typing

Genomic DNA was extracted from peripheral blood using 'Easy Blood DNA prep' (A&A, Gdansk, Poland) according to the original protocol. β<sub>2</sub>-ADR polymorphisms in positions: –47, –20, 46, 79, 252 (numbers refer to the first nucleotide of start codon as +1) were determined by direct sequencing of PCR products obtained with the following primers: 5'-CTG AAT GAG GCT TCC AGG CGT-3' and 5'-ACA ATC CAC ACC ATC AGA AT-3'. The 584-bp PCR fragments were purified with a commercial kit (Qiagen, Valencia, CA, USA) and sequenced using fluorescently labelled dye terminators technique ('Big-Dye Terminator Cycle Sequencing', Applied Biosystems, Foster City, CA, USA) in ABI Prism 310 capillary sequencer (Applied Biosystems). Ambiguous samples were sequenced in both directions.

### Statistical analysis

The frequencies of β<sub>2</sub>-adrenoceptor polymorphic alleles/haplotypes/genotypes in studied groups were compared with two-sided Fisher's exact test. Odds ratio (OR) was calculated as the cross product in a 2 × 2 table within 95% confidence intervals (CI). For the comparison of quantitative traits of log-total IgE levels, skin prick tests and spirometry results ANOVA, Mann–Whitney and Student's *t*-tests were used, as appropriate. To correct for incidental significance, the *P*-value was multiplied by the number of alleles compared (*p*<sub>cor</sub>; Bonferroni correction).

## Results

The β<sub>2</sub>-ADR genotype frequencies for all tested polymorphic loci (–47 T/C, –20 T/C, 46 A/G, 79 C/G and 252 G/A) in patients with grass allergy and in nonatopic controls are shown in Table 2. The number of individuals

Table 2. Genotypic frequencies of  $\beta_2$ -adrenergic receptor loci in groups of studied patients ( $n = 110$ ) and nonatopic controls ( $n = 70$ )

| Genotype | Patients, <i>N</i> (%) | Nonatopic controls, <i>N</i> (%) |
|----------|------------------------|----------------------------------|
| -47 TT   | 46 (41.8)              | 32 (45.7)                        |
| TC       | 46 (41.8)              | 26 (37.1)                        |
| CC       | 18 (16.4)              | 12 (17.2)                        |
| -20 TT   | 55 (50.0)              | 36 (51.4)                        |
| TC       | 39 (35.4)              | 24 (34.3)                        |
| CC       | 16 (14.6)              | 10 (14.3)                        |
| 46 AA    | 25 (22.7)              | 14 (20.0)                        |
| AG       | 43 (39.1)              | 27 (38.6)                        |
| GG       | 42 (38.2)              | 29 (41.4)                        |
| 79 CC    | 47 (42.7)              | 26 (37.1)                        |
| CG       | 44 (40.0)              | 26 (37.1)                        |
| GG       | 19 (17.3)              | 18 (25.8)                        |
| 252 GG   | 62 (56.4)              | 46 (65.6)                        |
| GA       | 37 (33.6)              | 18 (25.8)                        |
| AA       | 11 (10.0)              | 6 (8.6)                          |

possessing each of the potential genotypes at each locus was consistent with Hardy–Weinberg equilibrium. There was no significant difference in genotype distribution between patients with grass allergy and nonatopic control subjects. To eliminate ambiguity in haplotype estimation based on heterozygotic subjects, for analysis of haplotypes only homozygotic genotypes were used. In all studied subjects eight haplotypes were identified (Table 3). Interestingly, three the most common haplotypes (1–3) represent 92% of the analysed homozygotic cohort. These three haplotypes contain the most commonly tested haplotypes constructed from loci 46 A/G and 79 C/G (encoding amino acids in position 16 Arg/Gly and 27 Gln/Glu, respectively) as: 16 Arg/27 Gln; 16 Gly/27 Gln; 16 Gly/27 Glu. We did not observe a haplotype representative for 16 Arg/27 Glu among studied subjects. Strong linkage disequilibrium phenomenon between analysed SNPs is present, however, no individual SNP adequately predicts complex haplotypes. But when two loci haplotypes (46 + 79) were tested for possible power of prediction of complex haplotype, more than 90% linkage was found with other polymorphic loci tested (Table 4).

Table 3. Frequencies of identified  $\beta_2$ -adrenergic receptor haplotypes, haplotypes were determined from homozygotic genotypes in all studied subjects ( $n = 75$ )

| Alleles   | -47 T/C | -20 T/C | 46 A/G | 79 C/G | 252 G/A | %     |
|-----------|---------|---------|--------|--------|---------|-------|
| Haplotype |         |         |        |        |         |       |
| 1         | T       | T       | A      | C      | G       | 42.67 |
| 2         | C       | C       | G      | G      | G       | 32.00 |
| 3         | T       | T       | G      | C      | A       | 17.33 |
| 4         | T       | T       | G      | G      | G       | 2.67  |
| 5         | T       | T       | A      | C      | A       | 1.33  |
| 6         | T       | T       | G      | C      | G       | 1.33  |
| 7         | C       | T       | G      | C      | A       | 1.33  |
| 8         | C       | T       | A      | C      | G       | 1.33  |

Table 4. Association between haplotypes of the polymorphisms in positions 46 and 79 and other polymorphisms tested, associations constructed from homozygotic genotypes

| Nucleotide position | Haplotypes              |                         |                         |
|---------------------|-------------------------|-------------------------|-------------------------|
|                     | 46 A/79 C, <i>N</i> (%) | 46 G/79 G, <i>N</i> (%) | 46 G/79 C, <i>N</i> (%) |
| -47 T               | 33 (97)                 | 2 (8)                   | 14 (93)                 |
| -47 C               | 1 (3)                   | 24 (92)                 | 1 (7)                   |
| -20 T               | 34 (100)                | 2 (8)                   | 15 (100)                |
| -20 C               | 0                       | 24 (92)                 | 0                       |
| 252 G               | 33 (97)                 | 26 (100)                | 1 (7)                   |
| 252 A               | 1 (3)                   | 0                       | 14 (93)                 |

There was no significant association between lung function measurements (forced vital capacity [FVC], FEV<sub>1</sub>) and  $\beta_2$ -ADR polymorphic loci. There was also no evidence of association between  $\beta_2$ -ADR alleles/genotypes/haplotypes and asthmatic phenotype, when group of patients with asthma/rhinitis and rhinitis only as well as nonatopic controls were compared (data not shown). When the patients with asthma/rhinitis and patients with rhinitis only were compared considering age, sex, smoking status, total serum IgE levels and specific IgE responses to grasses and other inhaled allergens analysed by skin prick tests, no significant differences were found between both groups. The only anticipated difference apart from differentially manifested symptoms, was bronchodilator response to salbutamol. A significantly higher ( $P < 0.0001$ ) response to bronchodilator was observed in patients having asthma (mean  $\Delta$ FEV<sub>1</sub> = 10.72  $\pm$  4.58) when compared to patients with rhinitis only (mean  $\Delta$ FEV<sub>1</sub> = 3.74  $\pm$  3.01). Bronchodilator response as qualitative variable in regards to  $\beta_2$ -ADR genotypes was examined using ANOVA. Significantly higher ( $p_{\text{cor}} = 0.0045$ ) response was observed only in patients with homozygotic genotype 46 A/A (mean  $\Delta$ FEV<sub>1</sub> = 10.51  $\pm$  5.52) in comparison with respective homo- (mean  $\Delta$ FEV<sub>1</sub> = 6.34  $\pm$  4.81) and hetero-zygotes (mean  $\Delta$ FEV<sub>1</sub> = 6.03  $\pm$  4.65). There was no significant difference in bronchodilator response when  $\beta_2$ -ADR haplotypes were analysed, although patients with haplotype (1) (containing 46 A allele) had the highest mean response (mean  $\Delta$ FEV<sub>1</sub> = 9.6  $\pm$  4.57) in comparison with other haplotypes tested (haplotype 2: mean  $\Delta$ FEV<sub>1</sub> = 7.37  $\pm$  6.25; haplotype 3: mean  $\Delta$ FEV<sub>1</sub> = 6.92  $\pm$  4.10). Interestingly, when group of patients with asthma/rhinitis and rhinitis only were examined separately for bronchodilator response in regards to  $\beta_2$ -ADR genotypes, significantly higher response in homozygotes 46 A/A (mean  $\Delta$ FEV<sub>1</sub> = 7.08  $\pm$  1.79;  $p_{\text{cor}} = 0.001$ ) when compared with hetero- ( $\Delta$ FEV<sub>1</sub> = 2.89  $\pm$  2.14) and 46 G/G homo-zygotes ( $\Delta$ FEV<sub>1</sub> = 3.12  $\pm$  2.29), was observed only in group of patients with rhinitis only.

The results of ANOVA for the relationship between each genotype for all SNPs and total serum log-IgE are shown in Table 5. Significantly higher total IgE levels were observed in homozygotic carriers of 46 A/A (mean log-IgE =  $2.54 \pm 0.38$ ;  $p_{\text{cor}} = 0.0015$ ) and 79 C/C (mean log-IgE =  $2.46 \pm 0.37$ ;  $p_{\text{cor}} = 0.003$ ) genotypes. When  $\beta_2$ -ADR haplotypes were analysed (Fig. 1), significantly higher total IgE levels (mean log-IgE =  $2.64 \pm 0.34$ ;  $p_{\text{cor}} = 0.0005$ ) were observed in patients having haplotype (1) (–47 T/–20 T/46 A/79 C/252 G). No significant association was observed between tested SNPs and total IgE levels in nonatopic control group.

Table 5. The genotypic association of  $\beta_2$ -adrenergic receptor loci with total serum immunoglobulin E (IgE) in group of atopic patients ( $n = 110$ ) assessed by ANOVA

| Genotype | Mean log-IgE $\pm$ SD | $p_{\text{cor}}$ |
|----------|-----------------------|------------------|
| –47 TT   | $2.35 \pm 0.41$       | NS               |
| TC       | $2.27 \pm 0.35$       |                  |
| CC       | $2.18 \pm 0.51$       |                  |
| –20 TT   | $2.36 \pm 0.16$       | NS               |
| TC       | $2.29 \pm 0.12$       |                  |
| CC       | $2.13 \pm 0.25$       |                  |
| 46 AA    | $2.54 \pm 0.38$       | 0.0015           |
| AG       | $2.32 \pm 0.34$       |                  |
| GG       | $2.14 \pm 0.41$       |                  |
| 79 CC    | $2.46 \pm 0.37$       | 0.003            |
| CG       | $2.21 \pm 0.35$       |                  |
| GG       | $2.10 \pm 0.45$       |                  |
| 252 GG   | $2.31 \pm 0.16$       | NS               |
| GA       | $2.32 \pm 0.16$       |                  |
| AA       | $2.10 \pm 0.10$       |                  |

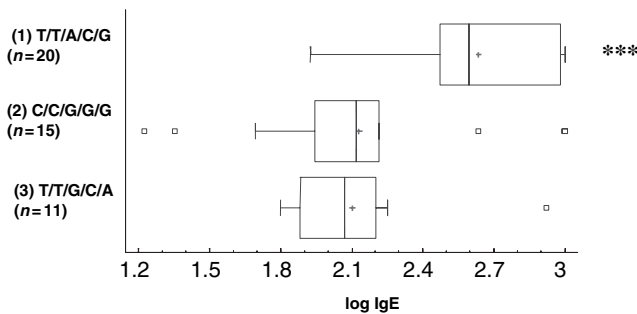


Figure 1. Comparison of total serum immunoglobulin E (IgE) levels (log-IgE) related to  $\beta_2$ -adrenergic receptor ( $\beta_2$ -ADR) haplotypes. Data are presented as a box and whisker plot with the central box covering the middle 50% of the data, the line through the box represents the median, the cross represents the mean and the whiskers the range. The outliers are shown as single points beyond the whiskers. Haplotypes are presented as nucleotides at positions: –49, –20, 46, 79, 252. \*\*\* $p_{\text{cor}} = 0.0005$ ; ANOVA between genotypes.

## Discussion

This is the first  $\beta_2$ -ADR association study in patients with grass allergy and the second in the literature (18) where DNA sequencing method was used to determine multiple  $\beta_2$ -ADR SNPs within particular haplotypes. In our Caucasian population we identified eight haplotypes, of these three (1–3) occurred with higher frequencies, representing more than 90% of analysed cohort. Drysdale et al. (18) using also DNA sequencing technology described 12 haplotypes constructed from 13 SNPs in  $\beta_2$ -ADR gene. Interestingly, three of them, fully corresponding to our haplotypes, covered nearly 95% of studied Caucasian population. Similar three major haplotypes were observed in children from CAMP study (6). Thus, it is very probable that most of Caucasian  $\beta_2$ -ADR genotypes contain mainly these haplotypes. Many studies concerning  $\beta_2$ -ADR polymorphisms were concentrated on two common SNPs at position 46 A/G and 79 C/G (encoding amino acids in positions 16 Arg/Gly and 27 Gln/Glu, respectively). These three, the most frequent haplotypes are representative for the following forms of  $\beta_2$ -ADR: 16 Arg/27 Gln (haplotype 1); 16 Gly/27 Glu (haplotype 2); 16 Gly/27 Gln (haplotype 3). We did not find a haplotype representative for 16 Arg/27 Glu among subjects studied, similarly to previous observations (14, 18). No individual  $\beta_2$ -ADR SNP adequately predicted complex haplotype. However, when two loci (46 and 79) haplotypes were constructed and tested in our population for prediction of other SNPs, more than 90% linkage was observed. This suggest that for some application it could be enough to identify haplotypes constructed from these two loci instead of typing all known SNPs, to predict full  $\beta_2$ -ADR haplotypes and genotypes at the acceptable level of confidence.

One of the phenotypes associated with  $\beta_2$ -ADR polymorphisms is bronchodilator response to  $\beta_2$ -agonists. In grass allergy patients we observed a significantly higher bronchodilator response to salbutamol in homozygotic subjects 46 A/A (16 Arg/Arg). No such relationship was identified when  $\beta_2$ -ADR haplotypes were analysed, although patients having homozygotic haplotype 1 had the highest bronchodilator response, but the difference did not reach statistical significance. Interestingly, when response to bronchodilator was analysed in groups of patients stratified by asthmatic phenotype, only in patients with rhinitis only, the significant difference was still present. Our observations confirm and extend those of Martinez et al. (24) who reported that asthmatic as well as nonasthmatic children homozygous for 16Arg are five times more likely to respond to albuterol in comparison with homozygotes for 16Gly and heterozygotes. The bronchodilator response is known to exhibit significant interindividual variation (25). This results suggest that in subjects homozygous for 16Arg genetically determined higher response to salbutamol might be independent of the factors related to asthmatic phenotype-like airway inflammation and bronchial hyperresponsiveness. It

might also explain a lack of association between  $\beta_2$ -ADR SNPs and bronchodilator response observed in asthmatic population, where enhanced inflammatory processes significantly influence responsiveness to  $\beta_2$ -agonist, hiding at the same time weaker expressed genetic predisposition (9). All these data fully support a so-called 'dynamic model' of  $\beta_2$ -ADR receptor kinetics proposed by Liggett (26). It has been suggested that endogenous catecholamines actively down-regulate  $\beta_2$ -ADR expression, thus 16Gly variant of receptor (a form more susceptible to down-regulation) would be down-regulated to a greater extent than 16Arg. This model would predict that the baseline bronchodilator response to exogenous agonist would be most apparent in 16Arg subjects, because their receptors have been endogenously less down-regulated. It would predict also the tachyphylactic effect of regular exposure to exogenous  $\beta_2$ -agonists in 16Arg asthmatics, as observed in the study of Israel et al. (9). We did not observe significant relation between  $\beta_2$ -ADR haplotypes and bronchodilator response, differently to Drysdale et al. (18) who found that response to albuterol in asthmatics was significantly related to haplotypes but not to individual SNPs. However, in the study of Silverman et al. (6) SNP +46 demonstrated association with bronchodilator response at both, single polymorphism and haplotype levels. These discrepancies could arise from difference in haplotype construction between these studies and number of SNPs typed. Another explanation might be related to clinically different population studied. In our case we decided to select a clinically well defined population of grass allergy patients, with symptoms related to single pathomechanism of allergic inflammation caused by grass allergens, less heterogeneous than most asthmatic populations and in that way potentially more suitable for genetic association study. Another important issue is a question of functional effects of  $\beta_2$ -ADR individual SNPs and haplotypes. Whilst the association of the amino acid 16 and 27 polymorphisms with altered receptor down-regulation profiles is proven the mechanism underlying these effects is uncertain. Based on the current studies it could be speculated that in the case of bronchodilator response it is rather a single locus in position 16 that determines predisposition for higher response to bronchodilators, than a full  $\beta_2$ -ADR haplotype (containing this locus). But before elucidation of a true mechanism causing this effect, it will be difficult to fully prove this hypothesis.

Another important observation from this study concerns association of  $\beta_2$ -ADR haplotype with total serum IgE levels. Significantly higher total IgE levels were found in subjects with haplotype 1 in comparison with other haplotypes tested. In addition, significant associa-

tions between total IgE and  $\beta_2$ -ADR polymorphisms were found when single locus genotypes were analysed. Patients homozygotic for 16 Arg and 27 Gln had significantly higher levels of IgE. Our findings are in agreement with observation of Dewar et al. (27) who found significant association and linkage between total IgE and 27 Gln receptor variant. Positive association of haplotype containing 27Gln with increased levels of IgE was also found in CAMP children (6). However, other studies analysing relation between  $\beta_2$ -ADR polymorphisms and total IgE have brought negative results (15, 17). In the case of total serum IgE regulation, in contrast to bronchodilator response, it is  $\beta_2$ -ADR haplotype not single SNP that determines genetic predisposition influencing this phenotype. Therefore, mainly haplotypic analysis of  $\beta_2$ -ADR association with total IgE levels may show a true relationship. This finding would support theory that there is another locus important in the control of IgE levels nearby on chromosome 5q31 which is in strong linkage disequilibrium with  $\beta_2$ -ADR locus, inherited as extended haplotype. Several studies have shown significant linkage between genetic markers in this region and total serum IgE (28, 29). Another explanation for this positive observation may be related to the low mean age of analysed groups in our and Silverman et al.'s (6) studies. It is known that heritability of IgE decreases with increasing age (30), thus decreasing the power to detect an association. In fact, Dewar et al. (27) observed association of  $\beta_2$ -ADR with total IgE in a study of younger population, but they did not find such an association in population with higher mean age (15). Because an overall contribution of  $\beta_2$ -ADR locus to the regulation of total IgE level is rather small, therefore the positive association could be lost due to large number of other factors influencing total IgE in older and less homogenous population.

It is clear that  $\beta_2$ -ADR polymorphisms are not genetic determinants of susceptibility to bronchial asthma. However several, consistent observations suggest that depending on examined asthmatic phenotypes either an individual  $\beta_2$ -ADR SNP or  $\beta_2$ -ADR haplotype may affect disease manifestation. Future studies are required to show whether these findings could be used in clinical applications and whether it will require typing of all known  $\beta_2$ -ADR polymorphisms to predict associated phenotypes.

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