

# Beta<sub>2</sub> adrenergic receptor gene restriction fragment length polymorphism and bronchial asthma

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## Abstract

**Background** - Beta<sub>2</sub> adrenergic dysfunction may be one of the underlying mechanisms responsible for atopy and bronchial asthma. The gene encoding the human β<sub>2</sub> adrenergic receptor (β<sub>2</sub>ADR) has recently been isolated and sequenced. In addition, a two allele polymorphism of this receptor gene has been identified in white people. A study was carried out to determine whether this polymorphism is functionally important and has any relation to airways responsiveness, atopy, or asthma.

**Methods** - The subjects studied were 58 family members of four patients with atopic asthma. Restriction fragment length polymorphism (RFLP) with Ban-I digestion of the β<sub>2</sub>ADR gene was detected by a specific DNA probe with Southern blot analysis. Airways responses to inhaled methacholine and the β<sub>2</sub> agonist salbutamol, the skin prick test, and serum IgE levels were also examined and correlated to the β<sub>2</sub>ADR gene RFLP. In addition, measurements of cAMP responses to isoproterenol in peripheral mononuclear cells were performed in 22 healthy subjects whose genotype for β<sub>2</sub>ADR was known.

**Results** - A two allele polymorphism (2.3 kb and 2.1 kb) of the β<sub>2</sub>ADR gene was detected in the Japanese population. Family members without allele 2.3 kb (homozygote of allele 2.1 kb) had lower airways responses to inhaled salbutamol than those with allele 2.3 kb. The incidence of asthma was higher in those without allele 2.3 kb than in those with allele 2.3 kb. The β<sub>2</sub>ADR gene RFLP had no relation to airways responses to methacholine and atopic status. cAMP responses in peripheral mononuclear cells of the subjects without allele 2.3 kb tended to be lower than those of the subjects with allele 2.3 kb. **Conclusions** - These results suggest that Ban-I RFLP of the β<sub>2</sub>ADR gene may have some association with the airways responses to β<sub>2</sub> agonists and the incidence of bronchial asthma.

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**Keywords:** β<sub>2</sub> adrenergic receptor gene, restriction fragment length polymorphism (RFLP), bronchial asthma, airways responsiveness.

Genetic and environmental factors are known to affect the incidence and severity of asthma. In 1968 Szentivanyi proposed the "β adrenergic theory" of asthma - that is, that asthma results from reduced function of the β adrenergic system.<sup>1</sup> Since then many studies have investigated this theory. Systemic responses to catecholamines - such as a rise in blood levels of sugar, free fatty acid, lactate, pyruvate, and plasma cyclic AMP (cAMP) - have been shown to be reduced in asthmatic patients.<sup>2</sup> Reduced cAMP responses of lymphocytes from asthmatic patients have been also reported.<sup>3,4</sup> In addition, two studies have shown that the relaxant potencies of β stimulants were significantly attenuated in bronchial preparations from asthmatic patients.<sup>5,6</sup> These studies support the "β adrenergic theory". However, responses to β stimulants have also been shown to be affected significantly by systemic or local treatment with β agonists and by viral respiratory tract infections.<sup>7,8</sup> The presence of these factors, which modify β adrenergic functions, has made it difficult to confirm a clear relation between abnormal β adrenergic functions and asthma.

The gene encoding the human β<sub>2</sub> adrenergic receptor (β<sub>2</sub>ADR) has recently been cloned and sequenced.<sup>9,10</sup> It is an intronless gene that has been localised to q31q32 on chromosome 5.<sup>9,11</sup> This locus is close to the locus called "5q cluster" in which the genes for IL-3, IL-4, IL-5, and granulocyte-macrophage colony stimulating factor (GM-CSF) are located. In addition, DNA polymorphism of the β<sub>2</sub>ADR with Ban-I digestion has been examined and a two allele polymorphism has been reported in 20 unrelated white people in North America.<sup>12</sup>

We have therefore examined the β<sub>2</sub>ADR gene restriction fragment length polymorphism (RFLP) in a Japanese population of 58 family members of four atopic asthmatic patients and investigated the relation to atopic state, non-specific bronchial hyperresponsiveness to methacholine, airways responses to a β<sub>2</sub> agonist, and the incidence of physician diagnosed asthma.

## Methods

### SUBJECT SELECTION

A total of 58 subjects, family members of four patients with bronchial asthma, were examined. They comprised three generations with 17, 12, 13, and 16 subjects from each family. The

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average age of the subjects was 34 years (range 7–80). The families were studied from June to August 1990 and no members were diagnosed as having a bronchial infection within two weeks of the investigation. All investigations were performed in the morning. The criteria for the diagnosis of asthma were cough, dyspnoea, wheeze on chest auscultation, and an improvement in forced expiratory volume in one second ( $FEV_1$ ) by an increment of 20% or more after administration of aerosol bronchodilator or other asthma treatment.

#### QUESTIONNAIRE

The subject's clinical history was taken by a physician who used a modified form of the American Thoracic Society respiratory questionnaire<sup>13</sup> which included a full history related to respiratory diseases. They were also asked about seasonal variations and the frequency of the symptoms, precipitants such as dust exposure, exercise, cold air and infections, and whether their symptoms had been diagnosed as bronchial asthma by a doctor. The subject's smoking history was recorded.

#### EXAMINATION FOR ATOPY

Skin prick testing for 26 common airborne allergens including house dust mite, pollens, fungus, and cat dander (Torii, Tokyo) was performed with a negative control. The results were read after 15 minutes and the mean weal diameters were recorded. The total serum IgE was measured by a solid phase immunoassay (RIST) and antigen specific IgE by the multiple antigen simultaneous test (MAST) for 16 common airborne allergens. When the subjects had at least one of the following three criteria they were assigned as atopic: one or more positive skin prick tests; a total IgE level of more than 250 U/ml; and one or more positive specific IgE measurements as identified by the MAST.

#### AIRWAYS RESPONSIVENESS TO METHACHOLINE AND $\beta_2$ AGONIST

Airways responses to methacholine and salbutamol were performed with the Astograph

(Chest, Japan) developed by Takishima *et al.*<sup>14</sup> Briefly, aerosols of methacholine in doubling concentrations (0.049–100 mg/ml) were continuously inhaled through the mouth by tidal breathing for one minute each. The aerosol was generated by a micronebuliser (Bird) with an output of 0.15 ml/min, and the size of particles ranged from 0.5 to 4.0  $\mu$ m. Bronchial responsiveness was measured from the change in respiratory resistance (Rrs, cm H<sub>2</sub>O/l/s) obtained by the forced oscillation technique. As shown in fig 1, Rrs was continuously and simultaneously measured during inhalation of the aerosols. When Rrs reached twice the initial value a bronchodilator aerosol (2% salbutamol) was inhaled for four minutes. Rrs was also measured continuously during this period. All the subjects were examined during quiet breathing.

The cumulative methacholine dose required to reduce respiratory conductance ( $Gr_s = 1/Rrs$ , l/s/cm H<sub>2</sub>O) by 35% of the baseline value ( $PD_{35}$ ) was determined.  $PD_{35}$  was calculated in terms of a unit (U) defined as one minute inhalation of 1 mg/ml methacholine. The  $PD_{35}$  determined by this method has a good correlation with the  $PD_{35}$  determined by measurement of airways resistance ( $R_{aw}$ ) by body plethysmography.<sup>15</sup> The  $\log(PD_{35})$  of 32 normal volunteers (average age 32 years) was also determined by the same method. Family members whose  $\log(PD_{35})$  was less than the mean – 2SD of that in normal volunteers (<1.39 U) were considered as having airways hyper-responsiveness to methacholine. Responses to the  $\beta_2$  agonist were evaluated by (1) the linear slope of the  $Gr_s$  versus time curve [ $\beta$ -S $Gr_s$  ( $= \Delta Gr_s / \Delta t$ ), l/s/cm H<sub>2</sub>O/min], and (2) the absolute amount of the increase in  $Gr_s$  (abs- $\beta$ - $Gr_s$ , l/s/cm H<sub>2</sub>O) after four minutes inhalation of salbutamol (fig 1). The  $\beta$ -S $Gr_s$  and abs- $\beta$ - $Gr_s$  values of normal volunteers were also determined by the same method. Family members whose  $\beta$ -S $Gr_s$  and abs- $\beta$ - $Gr_s$  values were less than the mean – 2SD of that in normal volunteers (<0.02 l/s/cm H<sub>2</sub>O/min and <0.035 l/s/cm H<sub>2</sub>O, respectively) were considered as having airways hyporesponsiveness to a  $\beta$  agonist. When functional antagonism was considered it is possible that the greater the methacholine response, the less the salbutamol response. To eliminate this we also calculated a value in which  $\beta$ -S $Gr_s$  was divided by the minimum  $Gr_s$  value ( $Gr_{s, min}$ ) just before salbutamol was inhaled [ $\beta$ -S $Gr_s$ / $Gr_{s, min}$  (1/min)].

#### DNA EXTRACTION AND HYBRIDISATION

High molecular weight DNA was extracted from peripheral blood leucocytes by the standard phenol extraction method,<sup>16</sup> digested with the restriction endonuclease Ban-I (Toyobo), electrophoresed in 0.7% agarose horizontal gel for 24 hours, and transferred onto nylon membrane (Hybond N<sup>+</sup>, Amersham, UK) by Southern blot. The membrane was hybridised in a mixture of 50% formamide, 0.1% Denhart's solution, 5 × SSPE (3.75 mol NaCl, 1.0 mol NaH<sub>2</sub>PO<sub>4</sub>, 0.1 mol EDTA, pH 7.4), 1.0%

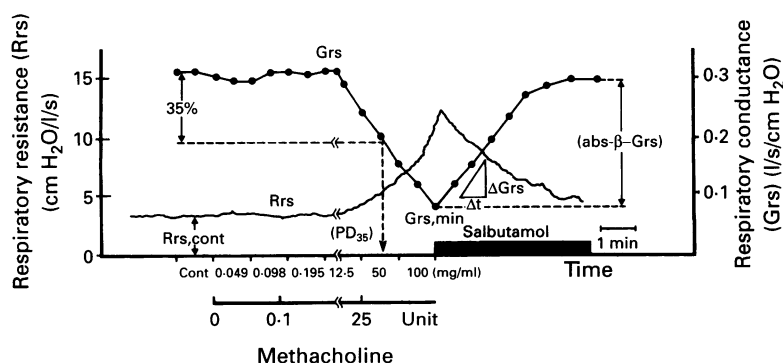


Figure 1 Analysis of typical dose-response curve for the airway responses to methacholine and salbutamol. With inhalation of incremental challenge of methacholine, Rrs increased curvilinearly. When Rrs reached twice the initial Rrs, inhalation of methacholine was stopped and salbutamol was inhaled. Parameters of airway responsiveness were measured by the cumulative dose of methacholine required to reduce  $Gr_s$  by 35% of baseline value ( $PD_{35}$ ), the linear slope of  $Gr_s$  versus time ( $\beta$ -S $Gr_s$  ( $= \Delta Gr_s / \Delta t$ )), and the absolute increase in  $Gr_s$  (abs- $\beta$ - $Gr_s$ ).

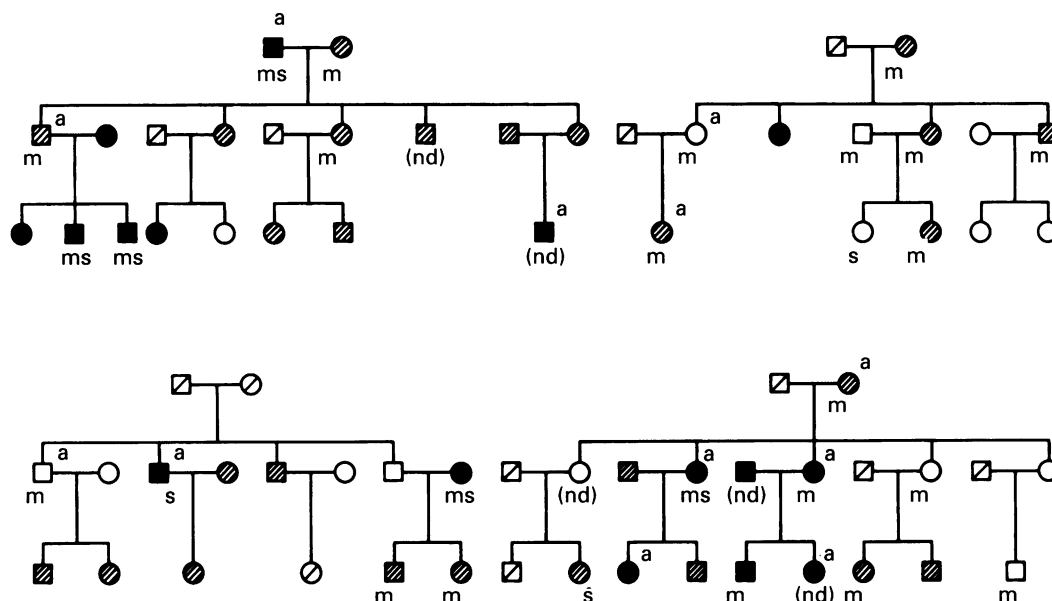


Figure 2 Family trees of the four families studied. Closed, slanted and open symbols = the genotypes (allele 2.1 kb homozygote, allele 2.1 kb and 2.3 kb heterozygote, and 2.3 kb homozygote, respectively). "m", "s", and "a" = phenotypes of hyperresponsiveness to methacholine and hyporesponsiveness to salbutamol and asthma, respectively. "nd" = subjects whose airway responses were not determined.

sodium dodecyl sulphate (SDS), 5% dextran sulphate, and 0.2 mg/ml sonicated salmon sperm with radiolabelled probe for  $\beta_2$ ADR gene (provided by Dr KU Lentens<sup>12</sup>). The size of the DNA probe is 2.6 kb. In addition to 1.2 kb  $\beta_2$ ADR structural gene, it also covers 1.0 kb of 5' side and 0.4 kb of 3' side of the structural gene. Autoradiography was performed overnight at  $-80^\circ\text{C}$ .

#### MEASUREMENT OF cAMP RESPONSES IN PERIPHERAL MONONUCLEAR CELLS

Twenty two healthy subjects, whose genotype for  $\beta_2$ ADR gene had been determined previously, were selected. Venous blood, 20 ml, was drawn from the antecubital vein of the subjects between 09.00 and 10.00 hours. Mononuclear cells were prepared by density gradient centrifugation in Ficoll-Hypaque (Pharmacia, USA). The cells were incubated with or without isoproterenol ( $10^{-8}$  to  $10^{-5}$  M) in the presence of theophylline (10 mM). After 20 minutes incubation the reaction was terminated by placing the tubes in boiling water for five minutes. cAMP was measured by radioimmunoassay (RIA) using a commercial kit (Yamasa, Japan).

#### DATA ANALYSIS

Segregation analysis was accomplished using the Devie's "singles" method.<sup>17</sup> Linkage analysis for atopy and methacholine or salbutamol airways responsiveness to the DNA polymorphism defined by the  $\beta_2$ ADR probe was made by the lod score method with the use of the LIPED computer program.<sup>18</sup> Airways responsiveness to methacholine ( $\log(\text{PD}_{35})$ ) and salbutamol ( $\beta$ -SGrs and abs- $\beta$ -Grs) were compared between the groups with two different allele patterns using the unpaired Student's

*t* test. The numbers of physician diagnosed asthmatic subjects were also compared between groups by the  $\chi^2$  method, a *p* value of  $<0.05$  being considered significant. Values are expressed as mean (SE).

## Results

#### STATUS OF ATOPY AND ASTHMA

As shown in fig 2, the four families had 17, 12, 13, and 16 subjects and the number of subjects judged to be atopic by our definition was 12, 8, 8, and 14 respectively. There were 12 asthmatic subjects (seven men) including four original patients in the 58 family members. Nine of the 12 asthmatic subjects were atopic. Five patients had no treatment, two were treated with inhaled  $\beta$  agonist on demand, three received oral and inhaled  $\beta$  agonists, and the remaining two patients were treated with oral and inhaled  $\beta$  agonists and inhaled steroids. The patients were classified as having mild to moderately severe asthma. Six were diagnosed by the respiratory specialists and the remaining six by the general physicians. An improvement in FEV<sub>1</sub> of 20% after administration of aerosol bronchodilator or other treatment was confirmed in six cases. Since the other six asthmatics had no symptoms for more than a month and had a normal FEV<sub>1</sub> at the time of examination, reversibility was not examined.

#### SEGREGATION OF AIRWAYS RESPONSIVENESS TO METHACHOLINE AND SALBUTAMOL, ATOPY AND BAN-I RFLP OF THE $\beta_2$ ADR GENE

Figure 3 shows the segregation of airways responsiveness to methacholine and RFLP of the  $\beta_2$ ADR gene in one of the four families studied. In each of the four extended families the transmission of hyperresponsiveness to methacholine occurred vertically. The number of

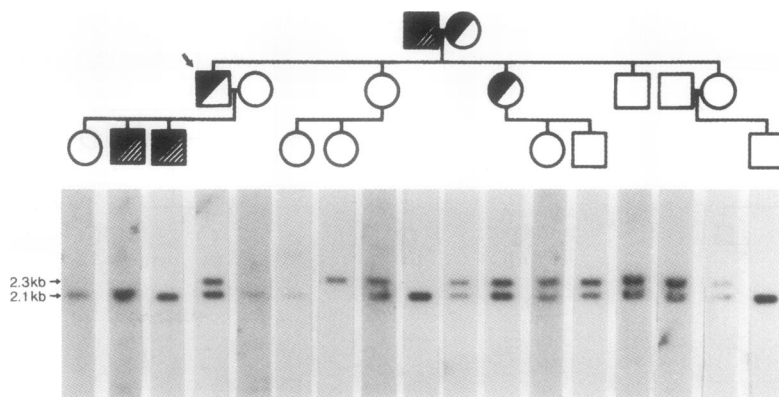


Figure 3 Segregation of airways hyperresponsiveness to methacholine and airways hyporesponsiveness to salbutamol and  $\beta_2$ ADR gene Ban-I RFLP. Closed and slanted marks represent hyperresponsiveness to methacholine and hyporesponsiveness to salbutamol, respectively. Arrow indicates the proband of this family.

Table 1 Association between airways responsiveness to methacholine and atopy

	Atopy (+)	Atopy (-)	Total
Hyperresponsiveness (+)	16 (44.4%)	8 (47.1%)	24
Hyperresponsiveness (-)	20 (55.6%)	9 (52.9%)	29
Total	36 (100%)	17 (100%)	53

$\chi^2 = 0.032$ ,  $p > 0.1$ .

subjects found to be hyperresponsive in each family were six of 17, seven of 12, four of 13, and seven of 16 members, respectively. The rate of the methacholine hyperresponsive children to all children from marriages with a single parent was not statistically different from the ideal value (50%) under the hypothesis of autosomal dominant inheritance. Table 1 shows that there was no significant relation between atopy and airways hyperresponsiveness to methacholine.

Two different alleles (2.3 kb and 2.1 kb) were detected with the  $\beta_2$ ADR gene probe (fig 3). Allele sizes and patterns were the same in each of the families. In linkage analysis we set the frequency of the alleles to 40% and 60% for alleles 2.3 kb and 2.1 kb, respectively, and assumed an autosomal dominant mode of inheritance with 10% putative bronchial hyperresponsive gene frequency and 98% penetrance for heterozygotes. There were no significant

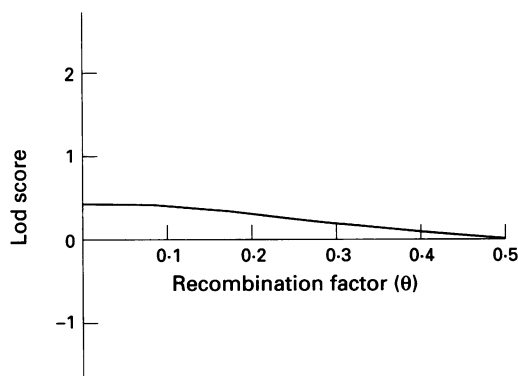


Figure 4 Lod scores for the linkage between hyporesponsiveness to inhaled salbutamol and  $\beta_2$ ADR gene Ban-I RFLP in all the families.

positive lod scores in any of the families. When the recombinant fraction  $\theta$  is 0 the lod score is less than  $-3.0$ . We also evaluated the lod score under an autosomal dominant mode of putative bronchial hyperresponsive gene frequency from 10% to 40% but could find no linkage in any family using this model. We also performed a linkage analysis between the  $\beta_2$ ADR gene RFLP and atopy but no significant positive lod scores were found in any of the families.

Figure 2 also shows the segregation of the airway responsiveness to  $\beta_2$  agonist (abs- $\beta$ -Grs) and RFLP of the  $\beta_2$ ADR gene in our families. In four families the mode of inheritance was compatible with autosomal recessive mode. Figure 4 shows the results of linkage analysis. We set the frequency of the alleles to 40% and 60% for alleles 2.3 kb and 2.1 kb, respectively, and assumed an autosomal recessive mode of inheritance with 10% putative bronchial hyporesponsive gene frequency and 98% penetrance for heterozygotes. When the recombinant fraction  $\theta$  is 0 the lod score is 0.4. Although the lod score was positive, it was not statistically significant.

#### ASSOCIATION BETWEEN ALLELES AND PARAMETERS OF AIRWAYS RESPONSIVENESS TO METHACHOLINE AND SALBUTAMOL

We successfully examined airways responses to methacholine in 53 subjects, including three who had no airways responsiveness to methacholine even at the highest concentration, and four subjects in whom salbutamol responses were not able to be determined due to coughing. Because of this, airways responses to salbutamol were examined in 46 subjects. For methacholine airways responses  $\log(\text{PD}_{35})$

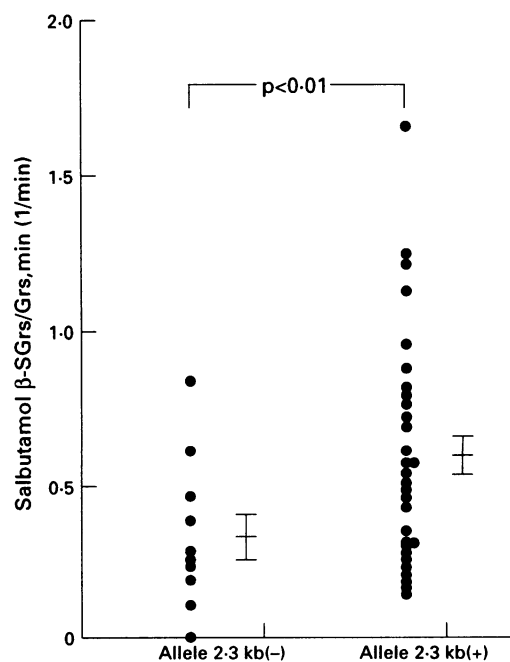


Figure 5  $\beta_2$ ADR gene Ban-I RFLP and airways responsiveness to salbutamol ( $\beta$ -SGrS/GrS, min). Subjects without allele 2.3 kb ( $n = 12$ ) had significantly smaller  $\beta$ -SGrS/GrS, min values than those with allele 2.3 kb ( $n = 34$ ) (mean (SE) 0.313 (0.07) and 0.603 (0.06), respectively,  $p < 0.01$ ).

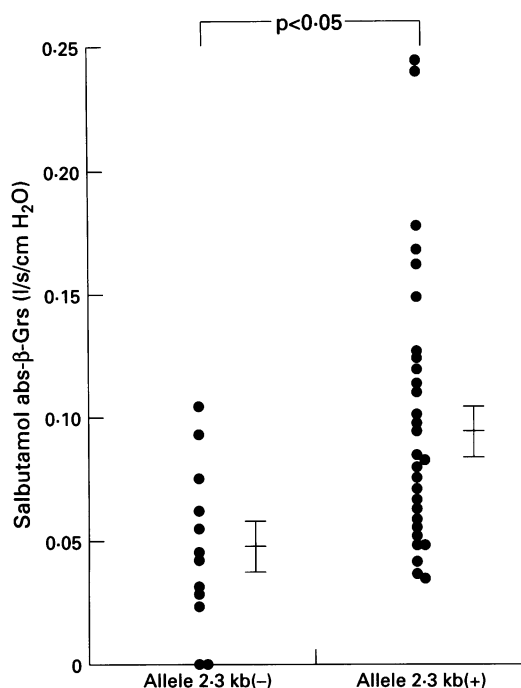


Figure 6  $\beta_2$ ADR gene *Ban-I* RFLP and airways responsiveness to salbutamol ( $\beta$ -abs-GrS). Subjects without allele 2.3 kb ( $n=12$ ) had significantly smaller abs- $\beta$ -GrS values than those with allele 2.3 kb ( $n=34$ ) (mean (SE) 0.049 (0.01) and 0.095 (0.01), respectively,  $p<0.05$ ).

values were 1.40 (0.11) U and 1.55 (0.17) U for subjects with allele 2.3 kb and those without 2.3 kb, respectively. There was no significant association between them. For airways responsiveness to  $\beta$  agonist subjects without allele 2.3 kb had significantly smaller  $\beta$ -SGrS values than those with allele 2.3 kb (0.022 (0.004) and 0.047 (0.005), respectively,  $p<0.05$ ). However, there was a trend towards correlation between  $\beta$ -SGrS and Grs, min ( $r=0.25$ ,  $p<0.1$ ). For this reason the corrected reactivity  $\beta$ -SGrS/GrS, min was considered to be a better index of airways response to  $\beta$  agonists. The  $\beta$ -SGrS/GrS, min values for the subjects without allele 2.3 kb and those with allele 2.3 kb were 0.313 (0.07) and 0.603 (0.06), respectively. The subjects without allele 2.3 kb had significantly smaller  $\beta$ -SGrS/GrS, min values than those with allele 2.3 kb (fig 5,  $p<0.01$ ). Figure 6 shows the relation between the presence of allele 2.3 kb and abs- $\beta$ -GrS. Subjects without allele 2.3 kb also had significantly smaller abs- $\beta$ -GrS values than those with allele 2.3 kb (0.049 (0.01) and 0.095 (0.01), respectively,  $p<0.05$ ). Even after the elimination from the analysis of five asthmatic subjects who received regular  $\beta$  stimulant treatment the result was the same

as above (abs- $\beta$ -GrS values: 0.047 (0.01) and 0.096 (0.01) for subjects without allele 2.3 kb and those with allele 2.3 kb, respectively,  $p<0.01$ ).

There were no significant correlations between  $\log PD_{35}$  and abs- $\beta$ -GrS or  $\beta$ -SGrS ( $r=-0.14$  and  $-0.20$ , respectively). Although the mean values for abs- $\beta$ -GrS (0.067 (0.024) and 0.085 (0.009), respectively) and  $\beta$ -SGrS (0.034 (0.018) and 0.041 (0.004), respectively) were lower in asthmatic than in non-asthmatic subjects, the differences were not significant.

#### RELATION BETWEEN ALLELES AND THE INCIDENCE OF BRONCHIAL ASTHMA AND WHEEZE

Table 2 shows the relation between the existence of allele 2.3 kb and the frequency of physician diagnosed asthma. In the 16 subjects without allele 2.3 kb seven (43.8%) had bronchial asthma. On the other hand, only five of the 42 subjects (11.9%) with allele 2.3 kb had bronchial asthma. The incidence of bronchial asthma in the subjects without allele 2.3 kb was significantly higher than that in the subjects with allele 2.3 kb ( $\chi^2=6.89$ ,  $p<0.01$ ). In addition, 11 of 16 (68.8%) subjects without allele 2.3 kb had experience of wheeze but only 13 of 42 (31.0%) subjects with allele 2.3 kb had experienced it. The incidence of wheeze in subjects without allele 2.3 kb was also significantly higher than that in the subjects with allele 2.3 kb ( $\chi^2=6.82$ ,  $p<0.01$ ).

#### cAMP RESPONSES IN PERIPHERAL MONONUCLEAR CELLS

Eight subjects were without allele 2.3 kb (homozygotes for allele 2.1 kb) and 14 were with allele 2.3 kb (five homozygotes for allele 2.3 kb and nine heterozygotes for allele 2.1 kb and 2.3 kb). The results are shown in fig 7. Baseline cAMP levels/ $10^6$  mononuclear cells were 39.2 (3.6) pmol and 50.3 (6.5) pmol, respectively for the subjects without and with allele 2.3 kb (no significant difference). However, in terms of maximum response, cAMP levels were 101.3 (12.1) pmol and 133.6 (13.1) pmol for the subjects without and with allele 2.3 kb, respectively, which showed borderline significance ( $p<0.06$ ).

#### Discussion

Previous studies have suggested a genetic predisposition to the development of bronchial asthma. Familial aggregation and the concurrent presence of atopy and bronchial asthma in identical twins have been reported.<sup>19,20</sup> In addition, Longo *et al*<sup>21</sup> reported a bimodal distribution of bronchial hyperreactivity, suggesting that there may be two phenotypes for bronchial hyperresponsiveness, thereby supporting the theory that this is, to some extent, controlled genetically.

Abnormal  $\beta$  adrenergic function seen in asthmatic subjects has been investigated as a factor relating to bronchial asthma and airways hyperresponsiveness. Several studies have shown that

Table 2 Association between  $\beta_2$ ADR gene *Ban-I* RFLP and the incidence of asthma

	Allele 2.3 kb (-)	Allele 2.3 kb (+)	Total
Asthma (+)	7 (43.8%)	5 (11.9%)	12
Asthma (-)	9 (56.2%)	37 (88.1%)	46
Total	16 (100%)	42 (100%)	58

$\chi^2=6.89$ ,  $p<0.01$ .

$\beta_2$ ADR =  $\beta_2$  adrenergic receptor; RFLP = restriction fragment length polymorphism; allele 2.3 kb (-) = homozygotes of allele 2.1 kb; allele 2.3 kb (+) = heterozygotes of allele 2.1 kb and 2.3 kb and homozygotes of allele 2.3 kb.

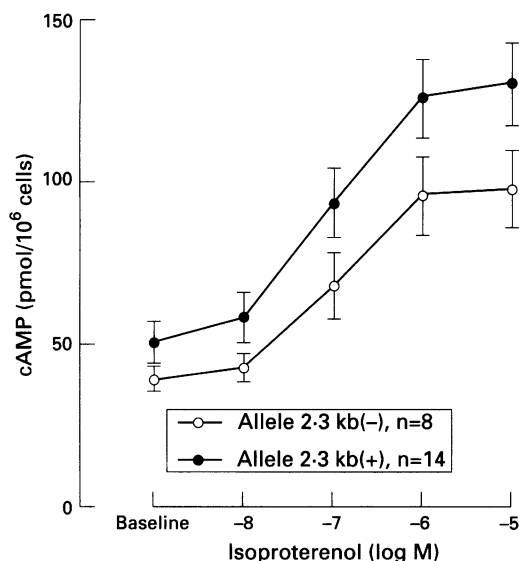


Figure 7  $\beta_2$ ADR gene Ban-I RFLP and cAMP responses of peripheral mononuclear cells to isoproterenol in normal healthy subjects. Allele 2.3 kb(-) = homozygotes of allele 2.1 kb; allele 2.3 kb(+) = heterozygotes of allele 2.1 kb and 2.3 kb and homozygotes of allele 2.3 kb.

the systemic effect of a  $\beta_2$  stimulant is blunted in asthmatic patients.<sup>2</sup> In lymphocytes obtained from asthmatic patients it has been reported that cAMP responses to  $\beta_2$  stimulation were significantly reduced.<sup>3,4</sup> In addition, receptor binding studies have revealed decreased  $\beta_2$  adrenergic receptor density without changes in affinity in drug free asthmatic subjects.<sup>22</sup> Although these facts suggest that  $\beta$  adrenergic dysfunction may be related to asthma and bronchial hyperresponsiveness, it is not clear whether these abnormalities are genetically determined. The gene encoding the human  $\beta_2$ ADR has recently been isolated and sequenced<sup>9,10</sup> and has been assigned to q31q32 on chromosome 5.<sup>9</sup> Lentjes *et al*<sup>12</sup> have shown that there is a DNA polymorphism in the  $\beta_2$ ADR gene in white people. These advances prompted us to study the relation between  $\beta_2$ ADR gene polymorphism and airways responses to methacholine and  $\beta_2$  adrenergic agonists and bronchial asthma.

This study revealed that there is also  $\beta_2$ ADR gene DNA polymorphism in the Japanese population. In each of the four extended families the transmission of the hyperresponsiveness to methacholine occurs vertically and this pattern of segregation suggests autosomal dominant inheritance. In linkage analysis, however, when the recombination fraction  $\theta$  is 0, the lod score is less than -3, suggesting the absence of linkage. There is also no significant association between this polymorphism and airways responses to methacholine. These results suggest that  $\beta_2$ ADR gene Ban-I RFLP have no significant relation to airways responsiveness to methacholine. Makino *et al*<sup>23</sup> reported that hypersensitivity of the bronchi to acetylcholine was correlated inversely with some  $\beta_2$  adrenergic responses to adrenaline. On the other hand, Okayama *et al*<sup>24</sup> also examined the relation between airways responses to methacholine and the  $\beta$  adrenergic blocking agent propranolol. They could not show a significant

correlation, suggesting instead that airways responses to methacholine and to  $\beta$  adrenergic blocking agents might be independent of each other. The results of our study were consistent with this. Examination of the relation between  $\beta_2$ ADR RFLP and airways responsiveness to propranolol is expected to provide additional useful information.

However, this study shows that this polymorphism may have a significant relation to airways responses to inhaled  $\beta_2$  agonists. When the recombination fraction  $\theta$  is 0, the lod score is 0.4 (fig 4) and no significant linkage could be found. However, the lod score for the airways responsiveness to a  $\beta_2$  agonist was positive, suggesting that more families need to be studied before a conclusion can be made as to whether there is a linkage between  $\beta_2$ ADR gene Ban-I RFLP and airways hyporesponsiveness to  $\beta_2$  agonists. Although the result of linkage analysis was inconclusive, we observed a significant association between this polymorphism and airways responses to inhaled  $\beta_2$  agonist. Family members without allele 2.3 kb (homozygotes of allele 2.1 kb) had a significantly lower airway response to a  $\beta_2$  agonist than those with allele 2.3 kb (homozygotes of allele 2.3 kb and heterozygotes of allele 2.1 kb and 2.3 kb) (figs 5 and 6). Even in normal healthy subjects, those without allele 2.3 kb had smaller  $\beta$ -SGRs/Gr<sub>s</sub>, min values than those with allele 2.3 kb (0.451 (0.07) and 0.717 (0.09), respectively,  $p < 0.05$ , unpublished data).

Fraser<sup>25</sup> showed, by using site directed mutagenesis of the human  $\beta_2$ ADR, that the replacement of cysteine with serine in some sites induces changes in the receptor number and affinity to different degrees. Strader *et al*<sup>26</sup> also showed that deletion of some amino acids from  $\beta_2$ ADR protein uncoupled the  $\beta_2$ ADR from stimulatory guanine binding protein (Gs). The results of this study suggest that alternation at the Ban-I recognition site of  $\beta_2$ ADR gene might be related in some way to the  $\beta_2$  receptor function. Reihnsaus *et al*<sup>27</sup> have recently examined mutations in the structural gene encoding for the  $\beta_2$ ADR in normal and asthmatic subjects and have found nine different point mutations at nucleic acid residues 46, 79, 100, 252, 491, 523, 1053, 1098, and 1239. Of these nine polymorphisms, four caused changes in the encoded amino acids at residues 16, 27, 34, and 164. The Ban-I recognition site coincided with nucleic acid residue 523 which did not cause a change in the encoded amino acid. From our study it was not possible to determine whether the Ban-I recognition site responsible for the RFLP detected in our study was due to the mutation at nucleic acid residue 523.

The fact that this polymorphism has a significant relation to an inhaled  $\beta_2$  agonist response may suggest the following possibilities. Since the size of the DNA probe used in this study is 2.6 kb, and also covers 1.0 kb of the 5' side and 0.4 kb of the 3' side of the  $\beta_2$ ADR structural gene, it is possible that the Ban-I recognition site responsible for the RFLP might be located outside the structural gene. At the 5' side of the structural gene there is some

1.6 kb long open reading frame. The existence of the promoter gene has been suggested in this area.<sup>10</sup> Another possibility is that there might be some linkage between mutation(s) in other structural or promoter gene DNA of  $\beta_2$ ADR and the Ban-I RFLP, even if the Ban-I recognition site coincides with nucleic acid residue 523 which causes no change in the encoded amino acid.

In measurement of cAMP in normal healthy subjects there was a trend towards reduced cAMP responses of peripheral mononuclear cells in subjects without allele 2.3 kb compared with those with allele 2.3 kb (fig 7). In addition, the incidence of physician diagnosed asthma and the number of subjects who had experienced wheeze were significantly higher among those without allele 2.3 kb than among those with it (table 2), suggesting that  $\beta_2$ ADR gene Ban-I RFLP may be related to the responsiveness to the  $\beta_2$  agonist and the incidence of bronchial asthma. Although the mean values for abs- $\beta$ -SGrS and  $\beta$ -SGrS were lower in asthmatic than in non-asthmatic subjects, however, the differences were not significant. This result, together with cAMP measurements, suggested that the difference in responses to  $\beta_2$  agonist related to the  $\beta_2$ ADR RFLP could explain only part of the genetic difference between asthmatic and non-asthmatic subjects. It is difficult to draw a clear conclusion from the results of the present study on the increased frequency of allele 2.1 kb homozygotes in the subjects with bronchial asthma as the number of family members and asthmatic subjects studied was small. A preliminary case-control study of unrelated asthmatic patients showed that homozygotes of allele 2.1 kb were found more commonly in patients with non-atopic asthma than in control subjects and in patients with atopic asthma.<sup>28</sup>

In summary, we have investigated the correlation between the  $\beta_2$ ADR gene RFLP and airways responses, atopy, and asthma. The results suggest that the  $\beta_2$ ADR gene Ban-I RFLP may have some relation to the  $\beta_2$  adrenergic airways responses and bronchial asthma.

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