

Association studies on β_2 -adrenoceptor polymorphisms and enhanced IgE responsiveness in an atopic population

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Summary

The β_2 adrenergic receptor 2 represents a cell surface receptor responsible for the binding of endogenous catecholamines and their exogenously administered agonists and antagonists, mediating their effects to the interior of the cell. On the basis of these functions, the observed association of two of its polymorphisms, Gly16 and Gln27, with nocturnal- and steroid-dependent asthma has been discussed. It has recently been suggested that Gln27 contributes to IgE variability in families with asthma.

The aim of this study was to investigate possible influences of the polymorphisms Arg16Gly and Glu27Gln on IgE levels in families recruited through an atopic index case without regard to the presence of clinical symptoms.

We employed linkage analysis in affected sibpairs characterized by elevated total IgE concentrations or sensitization to common inhalant allergens. Furthermore, we tested 258 children for association of any of the polymorphisms with enhanced IgE responsiveness.

We could find neither linkage at the locus 5q31 nor significant association of the polymorphisms with elevated total IgE concentrations or specific sensitization.

We conclude from our data that the polymorphisms Gln27Glu and Arg16Gly of the β_2 -adrenergic receptor do not play a significant role in the pathogenesis of enhanced IgE responsiveness in an atopic population in general.

Keywords: ADRB2, association, atopy, genetics, linkage, polymorphisms

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Introduction

The β_2 adrenergic receptor 2 (ADRB2) is a cell surface receptor responsible for the binding of endogenous catecholamines and their exogenously administered agonists and antagonists. Signal transduction is accomplished through guanine nucleotide regulatory binding proteins (Gs proteins) and stimulation of adenylyl cyclase. The ADRB2 is located on several cell types, including bronchial smooth muscle cells in the lung, mast cells and various other immune cells, epithelial cells and nerve terminals.

In 1968 Szentivanyi proposed the hypothesis that defects in the β -adrenergic pathway were involved in the development of bronchial asthma [1]. In the following years this

concept was supported by several findings of different scientific groups [2–5].

First evidence of polymorphisms of the ADRB2 gene being involved in steroid-dependent asthma came from Liggett and coworkers [6]. Although the prevalence of the polymorphisms Arg16Gly, Gln27Glu, Val34Met and Thr164Ile was the same in normal and asthmatic subjects, Gly16 was found to be more common in those asthmatics with steroid dependence and the need for immunotherapy [7]. Further studies revealed distinct changes of receptor functions in three of the four polymorphisms. The least common polymorphism, Ile164, resulted in reduced affinity for binding adrenaline as well as noradrenaline and decreased maximal stimulation of adenylyl cyclase on adrenaline binding was shown [8]. Based on this finding it has been speculated, that asthmatics bearing the Ile164 allele are less responsive to administered or endogenous β_2 -agonists.

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Gly16 and Glu27 did not show changes in the affinity to β_2 -agonists but displayed altered receptor down-regulation as induced by agonists. Thereby Gly16 showed enhanced, Glu27 significantly less agonist-induced down-regulation compared with the wild-type receptor [9]. Nocturnal asthmatics presented with a circadian down-regulation of their β_2 -adrenergic receptors compared with non-nocturnal asthmatics or normal individuals [10]. Gly16 was likely to enhance the down-regulation effect after midnight as well as reduced responsiveness on β_2 -agonist administration, although this remains hypothetical. In fact, Gly16 was shown to be more common in asthmatics with nocturnal complaints [9]. In addition, Gly16 is associated with a greater loss of bronchodilator response after long-term inhaled β_2 -agonist therapy [11,12]. Gly16 was strongly associated with bronchial hyperreactivity in atopic asthmatics [13], whereas Glu27 showed the opposite effect [14]. Regarding the effects on asthma, the ADRB2 polymorphisms are likely to affect the type and severity of asthma as well as the therapeutic response on β_2 -agonist administration.

Apart from the functional relevance and the disease-modifying character of the known ADRB2 polymorphisms, Dewar and coworkers were interested in a conceivable influence on the development of the atopic phenotype [15]. They found an association of the Gln27 polymorphism with elevated total IgE concentrations but not with bronchial asthma or specific sensitization to common inhalant allergens. However, the population presented in their study had been recruited through asthmatic index cases. In a further study by the same group no consistent association between ADRB2 polymorphisms and the risk of developing allergic disease could be shown [16].

It was our intention to investigate the Arg16Gly and Gln27Glu polymorphisms in an atopic population without regard to clinical symptoms. This was meant to reduce possible skewing of the underlying linkage and association effects due to the unspecificity of the clinical history of the probands as well as the influence of their therapeutic regimen.

Materials and methods

Families

One hundred and five nuclear families, partially recruited through a population-based study [17] and partially through the outpatients department of the University Children's Hospital Freiburg via serologically affected probands, are presented in this study. The populations comprised 105 sibpairs with elevated serum IgE concentrations and 123 sibpairs with specific sensitization to common inhalant allergens.

Individuals

Genotyping and association studies were performed on the 258 children of all 105 families. One hundred and sixty individuals (62%) showed total IgE concentrations above 100 kU/L and 186 (72%) specific sensitization to at least one inhalant allergen. Only 55 (21%) were affected by bronchial asthma.

Approval

The collection of serum and DNA material as well as the experiments were approved by the Ethical Committee of the University of Freiburg.

Phenotype analysis

Specific IgE was detected by enzyme-linked immunosorbent assays against two mixtures of grass pollens, *Dermaphagoides pteronyssinus*, *Dermatophagoides farinae*, cat dander and birch pollens (Magic Lite; ALK, Denmark). The cut-off point for a positive titre was 1.4 mL units [18]. Detectable IgE to one of the six groups of allergens or a positive skin prick test response to one of them were the criteria for 'any specific sensitization'. Measurement of total serum IgE was carried out by an enzyme allergosorbent test (Phadezym; Pharmacia, Uppsala, Sweden). The cut-off point for an elevated titre was 100 kU/L [19]. The diagnostic criterium 'Atopy' required any specific sensitization and/or an elevated total serum IgE, and corresponds to the 'combined definition'. Asthma was diagnosed on the basis of the patient's history of doctor's diagnosis and/or recurrent wheezing.

Genotype analysis

DNA was extracted from peripheral blood leucocytes following standard procedures and column purified (DNA midi kit; Qiagen, Germany). Genotypes were determined by polymerase chain reaction for the microsatellite marker D5S210 (Mfd122) flanking the ADRB2 gene and by a modified RFLP for the polymorphisms Arg16Gly and Gly27Glu.

Microsatellite analysis

Primer sequences for D5S210 were (5'→3') ATCTACTG-CAAGTGACGATC and CATCTCCCTACCATCTGACC. The amplified alleles were 114–130 bp in length (632).

PCR was carried out in a volume of 10 μ L containing 30 ng genomic DNA, 2.5 pmol of each primer, 0.5 U *Taq*-Polymerase (Pharmacia, Uppsala, Sweden) and 200 μ mol/L of each dNTP except 2.5 μ mol/L dCTP with the buffer

recommended by the supplier. (^{32}P)dCTP (200 $\mu\text{mol/L}$) was incorporated in the PCR reaction. After denaturation for 5 min at 94°C 35 cycles were carried out, each cycle consisting of 60 s at 94°C, 90 s at 62°C and 120 s at 72°C. The last synthesis step was extended to 7 min. The amplified products were resolved on 8% denaturing polyacrylamide gels and detected by autoradiography (Kodak X-omat XAR, 1.5–3 h). Allele sizes were determined by the use of known standards. The genotyping was performed by two investigators unaware of the phenotypes.

RFLP analysis

To amplify the target DNA in the polymorphic regions we used two oligonucleotide primer pairs. R16G: 5'-CCTTCTTGCTGGCACCCTCAT-3' and 5'-CCGTCTGCA GACGCTCGAAC-3'. Q27E: 5'-CGGACCACGACGTC ACCCAG-3' and 5'-CCGTCTGCAGACGCTCGAAC-3'. The PCR was carried out in a volume of 20 μL containing 30 ng DNA, 5 pmol of each primer, 0.06 U *Taq*-Polymerase (Pharmacia, Uppsala, Sweden) and 1 mmol/L of each dNTP with the buffer recommended by the supplier. After denaturation for 5 min at 94°C 35 cycles were carried out, each cycle consisting of 45 s at 94°C, 90 s at 60°C and 45 s at 72°C. The last synthesis step was extended to 10 min.

Phenotyping of the Arg16Gly polymorphism made use of a *Nco*I-site present in the allele coding for glycine (using a mutated PCR primer as indicated above). Restriction was performed in a volume of 10 μL containing 5 μL of the PCR-product, 2 U *Nco*I and the buffer recommended by the supplier for 60 min at 37°C. The fragments of 174 bp (Arg) or 157 bp and 17 bp (Gly) were resolved on a 12% polyacrylamide gel.

Phenotyping of the Gln27Glu polymorphism made use of a *Bst*NI-site present in the allele coding for glutamic acid (using a mutated PCR primer as indicated above).

Restriction was performed in a volume of 10 μL containing 5 μL of the PCR product, 2 U *Bst*NI and the buffer recommended by the supplier for 60 min at 60°C. The fragments of 75 bp and 66 bp (Gln) or 66 bp and 55 bp and 20 bp (Glu) were resolved on a 12% polyacrylamide gel.

One individual for each genotype was verified by direct genomic sequencing and used as a reference in the RFLP screening reactions.

Statistical analysis

All informative genotypes of affected sibpairs were included in this study. Sibpair analysis was performed on the basis of identity by descent (IBD) [20]. The observed distribution of shared and non-shared alleles in affected sibs was compared with the distribution expected under the hypothesis of non-linkage (25/50/25 or 50/50). Testing for significance made use of χ^2 goodness-of-fit testing.

The number of alleles for each polymorphism was counted in all 258 children of the study populations. To account for the individuals being siblings and to adjust for age and sex of the study population, regression analysis was applied. Logistic regression analysis and linear regression analysis for the explanation of the phenotypes 'specific sensitization to common inhalant allergens' treated as a dichotomous parameter and 'total serum IgE' treated as a continuous parameter on a logarithmic scale by the genotypes 'presence of the polymorphic allele' were performed. Analysis followed the method of Zeger and Liang to account for the correlation between sibs [21].

A *P*-value below 0.05 was considered significant. The factor 10^{Estimate} indicates the average IgE concentration in individuals bearing one of the polymorphisms compared with those bearing wild-type alleles. For example an estimate of 0.13 is equivalent to the average IgE level in individuals bearing one of the polymorphisms being 1.35 times the level in individuals bearing wildtype alleles.

Table 1. Affected sibpair analysis for the microsatellite marker D5S210. Given are the numbers of parental alleles shared in affected sibpairs based on three phenotype criteria. Sharing of alleles is shown for both parents as well as for maternal and paternal alleles separately

	Both parents						Mothers					Fathers				
	Alleles shared						Alleles shared					Alleles shared				
	<i>n</i>	0	1	2	χ^2	<i>P</i>	<i>n</i>	0	1	χ^2	<i>P</i>	<i>n</i>	0	1	χ^2	<i>P</i>
Specific sensitization	56	17	20	19	4.7	0.09	75	39	36	0.1	0.73	82	38	44	0.4	0.51
Serum IgE > 100	49	12	22	15	0.9	0.64	64	29	35	0.6	0.45	67	34	33	0.0	0.90
Combined definition	64	17	24	23	5.1	0.08	84	39	45	0.4	0.51	95	45	50	0.3	0.61

Table 2. Logistic regression analysis and linear regression analysis for the explanation of the phenotypes 'specific sensitization to common inhalant allergens' treated as a dichotomic parameter and 'total serum IgE' treated as a logarithmic linear parameter by the genotypes 'presence of the allele P503 or R576, respectively'. The positive parameter estimate indicates higher total IgE levels in individuals bearing the respective polymorphisms, it allows calculating the average IgE levels. The differences are not significant.

Polymorphism	Specific sensitization			log(IgE)		
	OR	CI (95%)	P-value	Parameter estimate	CI (95%)	P-value
Arg16Gly	1.42	0.66/3.04	0.37	0.13	-0.15/0.41	0.36
Gln27Glu	1.07	0.62/1.84	0.81	0.07	-0.08/0.23	0.35

Results

Employing sibpair linkage analysis with a microsatellite marker close to the ADRB2-gene we were unable to find significant sharing of parental alleles in sibpairs characterized by elevated total IgE concentrations or specific sensitization to common inhalant allergens (Table 1). Neither maternal nor paternal alleles were shared more often than expected by chance.

The genotypes for all children were determined in respect of the known polymorphisms Gln27Glu and Arg16Gly. Association of the genotypes with enhanced IgE responsiveness was tested separately for the two polymorphisms. No significant association was found (Table 2).

Discussion

It was the aim of this study to investigate the possible influence of the adrenoceptor- β_2 gene polymorphisms on the development of the atopic phenotype. This was of special interest as a direct influence of altered ADRB2 stimulation on increased IgE production had already been shown [22–24]. Furthermore, the polymorphism Gly 16 in the ADRB2 gene had been described as altering agonist-induced down-regulation of the receptor number on circulating neutrophils and lymphocytes [12]. This raises the question of whether the two polymorphisms Gln27Glu and Arg16Gly could be directly involved in enhanced IgE regulation.

We studied two populations, one recruited through a population-based study, the other through our outpatients department on the basis of immunological parameters but without regard to the presence of clinical symptoms.

Employing sibpair linkage analysis with a microsatellite marker close to the ADRB2-gene we were unable to find significant sharing of parental alleles in sibpairs characterized by elevated total IgE concentrations or specific sensitization to common inhalant allergens.

Although linkage analysis has been used successfully to find major genes, its power to detect genes with modest effect on the phenotype is rather low [25]. Thus, genes with a low relative risk for disease development might demand several thousands of affected sibpairs for significant linkage results. We could therefore not exclude a minor effect of the ADRB2 on the atopic phenotype on the basis of our negative linkage findings.

Much stronger results even in small numbers of individuals can be expected by association studies within a disease locus itself [25]. These studies include direct associations between particular polymorphic alleles within the gene and the phenotype under examination. Neither the Gln27Glu nor the Arg16Gly polymorphism showed any significant association with the two phenotypes studied. This makes a major effect of the ADRB2-gene on IgE regulation very unlikely.

In contrast with our findings, Dewar *et al.* found significant association between the Gln27Glu polymorphism and elevated total serum IgE levels [15]. We have four possible explanations for our study yielding contrary results: **1** Atopy represents a disease with major genetic influences, characterized by polygenic inheritance as well as genetic heterogeneity [26]. Furthermore, substantial racial differences in single gene influences have been found [27]. This could lead to the polymorphism Gln27Glu playing a minor role in the pathogenesis of atopy in our population, which might escape statistical significance.

2 The low level of significance in the study by Dewar *et al.* could result from a type I error or just reflect the effects of testing for multiple, dependent traits.

3 Linkage disequilibrium effects of ADRB2 and adjacent genes influencing immune responses might underlie positive association findings. In fact, several genes, which are involved in atopic reactions [28], have been mapped to the genetic region 5q31.1–33. These include IL4, IL5, IL9, IL13 and IRF1 among others. Although association studies narrow the chromosomal segment in which linkage disequilibrium

effects cause significant results, it might well count for the observed contradiction of Dewar's and our results.

4 Recently linkage disequilibrium has been shown between the amino acid 16 and 27 polymorphisms [16]. It is therefore difficult to study the effects of the two polymorphisms in isolation.

5 The method of recruitment of the families in Dewar's study was different from ours. Whereas they drew their individuals from 60 families multiplex for asthma, we recruited our families through an atopic individual presenting immunological markers for atopy (i.e. specific sensitization or elevated total IgE levels) but not necessarily clinical symptoms of atopy. In fact, only 55 of the 258 independent adults tested for association were asthmatic. Positive association results might therefore reflect primarily underlying genetic properties of the asthmatic phenotype.

6 It seems reasonable to assume that most of the individuals studied by Dewar *et al.* had been treated for a long time with ADRB2-agonists, as these are potent drugs and widely used in asthmatics. Long-term inhaled β_2 -agonist therapy in the presence of ADRB2 gene polymorphisms has been shown to be associated with an enhanced loss of bronchodilator response [11,12], which might be demonstrated by agonist-induced down-regulation of the receptor number. This in turn leads to enhanced IgE responsiveness, as has been shown by Coqueret *et al.* [23]. In fact fenoterol potentiates IL4-induced IgE production via the cAMP pathway [24]. The observed association of ADRB2 polymorphisms with elevated total IgE concentrations could therefore be interpreted as an effect of the usual therapeutic regimen of the asthmatic patients who made up the majority of the population studied by Dewar. If Gln27 was directly involved in altered IgE levels through the down-regulation of ADRB2 numbers on circulating immune cells, one should expect to see this effect especially in the absence of ADRB2-agonist therapy. However, we did not find any association in our atopic population, of which a minority only was treated with inhaled β_2 -agonists.

The aim of this study was primarily to analyse the effect of polymorphisms in the β_2 -adrenergic receptor on IgE levels exerted in atopic individuals of either phenotype. We conclude from our results that the polymorphisms Gln27Glu and Arg16Gly of the β_2 -adrenergic receptor *per se* do not play a significant role in the regulation of total IgE concentrations in an atopic population in general. We suggest carrying out further studies to compare possible effects of the above polymorphisms in asthmatic vs atopic but not asthmatic individuals. The potential influence of long-term inhaled β_2 -agonist therapy should be considered at the same time.

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