Genetic Polymorphisms of the Beta-2 Adrenergic Receptor in Israelis with Severe Asthma Compared to Non-asthmatic Israelis

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Abstract

Background: It has been argued that arginine replacement in locus16 (Arg16) of β_2 adrenergic receptor with glycin (Gly16) increases asthma severity, while glutamin replacement in locus 27 (Gln27) with glutamic acid (Glu27) decreases it. In addition, ethnic dependency of these polymorphisms has been described, but few studies investigated its relation to asthma severity in a non-anglosaxic population.

Objectives: To investigate non-anglosaxic ethnic influences on β_2AR polymorphisms and its correlations to asthma severity.

Methods: Sixty-six Israeli Jewish and Arab asthmatics who had near-fatal asthma and/or severe nocturnal asthma and/or steroid-dependency were investigated for genetic polymorphisms of β_2AR and compared to matched controls. The Jewish patients included both Ashkenazi (of European origin) and non-Ashkenazi (originating from the Middle East or North Africa). The results were compared with those of ethnically matched 113 non-asthmatic Israelis and non-asthmatic Anglo-Saxons described in the literature.

Results: We found no significant genetic differences between the asthmatics and their controls or between the various ethnic groups of our population. However, the prevalence of Glu27 was significantly lower in non-asthmatic Israelis compared to non-asthmatic Anglo-Saxons.

Conclusions: The genetic distribution of β_2AR polymorphisms in severe Israeli asthmatics is not different from that of non-asthmatic Israelis and therefore its clinical impact on asthma is probably minimal.

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The hypothesis that a defective beta-2 adrenergic receptor might be a primary causal abnormality in asthma was first proposed by Szentivanyi [1]. Several lines of evidence supported this hypothesis [2], but in the last two decades it was overshadowed by the emerging "inflammatory theory."

In 1987 Kobilka et al. [3] determined the structure and sequence of β_2AR, and 6 years later Reishause and co-workers [2] screened it for possible mutations. Of the several mutations discovered two are quite common (so-called polymorphisms) and were proved *in vitro* to affect the down-regulation of the β_2AR [4]. Replacement of the arginin in codon 16 (Arg16) with glycin (Gly16) (Arg16Gly) *in vitro* enhances the down-regulation of β_2AR and therefore may increase the sensitivity of the bronchial tree *in vitro*. On the other hand, replacement of glutamin in codon 27 (Gln27) with glutamic acid (Glu27) (Gln27Glu) reduces it and may therefore decrease bronchial smooth muscle sensitivity. Clinical investigations found no difference in the distribution of the polymorphisms between asthmatic subjects and non-asthmatics, but some authors have found a correlation between the frequency of Gly16 and the characteristics of severe asthma [2,5–8]. However, other investigators disagree with this conclusion [2,9]. In addition, Weir, Xie and Aynacioglu and their teams [9–11] found that polymorphisms of Gly16 and Glu27 are significantly less frequent in populations of non-western origin. Since the relation between these polymorphisms and asthma severity remains unclear, and since this issue has been investigated mainly in western populations, we investigated the frequency of β_2AR polymorphisms in non-asthmatic Israelis and Israelis with severe asthma. We chose to study asthmatics who had clinical attributes similar to those described in previous studies that investigated the clinical correlates of β_2AR polyphormisms, namely near fatal asthma, nocturnal asthma and corticosteroid dependency.

Materials and Methods

Population and asthma severity criteria

We investigated 66 Israelis with severe asthma. The diagnosis of asthma was determined according to criteria of the National Heart, Lung and Blood Institute [12]. The disease was defined as severe when the patient had at least one of the following three criteria: a) corticosteroid dependency, namely the need for oral corticosteroids continuously over a period of at least 6 consecutive months; b) nocturnal asthma attacks at least four nights a week over a period of at least 6 consecutive months, and c) at least one attack of near fatal asthma. We define near fatal asthma as an asthmatic attack requiring intubation for mechanical ventilation, or causing $PaCO_2$ greater than 50 mmHg, or an altered state of consciousness [13]. Of the 66 asthmatic subjects, 38 patients had a history compatible with atopy. Forty-three had a first-degree relative with

β_2AR = beta-2 adrenergic receptor

Table 1. Characteristics of our subjects with severe asthma

		NFA +NA +CSD	NFA +NA	NFA +CSD	NA +CSD	NFA	NA	CSD	All
No. of subjects		16	18	8	6	11	6	1	66
Demography									
Mean age		44	41	56	47	41	29	41	43
(range)		(9–73)	(21–57)	(16–66)	(28–65)	(19–71)	(21–46)		(9–73)
Male/Female		9/7	7/11	6/2	3/3	5/6	5/1	1/0	36
Clinical characteristics									
Disease duration in years		16	23	9	26	26	22	16	20
(range)		(1–48)	(1–42)	(2–20)	(12–45)	(1–66)	(1–27)		(1–66)
Total admissions >5		13	6	4	5	1	2	1	32
Smoking > 5 cigarettes/day		0	0	3	2	0	1	0	6
Ex–smoker		3	1	2	3	1	2	1	13
FEV_1									
FEV_1 % of	Before	52	56.5	52	63.5	58	60	31	56
predicted (range)	bronchodilator	(32–85)	(22–98)	(36–79)	(35–84)	(22–84)	(47–75)		(22–98)
FEV_1 % of	After	67	67	68	73.5	72	72	46	68
predicted (range)	bronchodilator	(42–97)	(37–96)	(62–84)	(54–104)	(30–102)	(59–82)		(30–104)

NFA = near fatal asthma, NA = nocturnal asthma, CSD = systemic corticosteroid dependency, FEV_1 = forced expiratory volume during 1 second

such a history. All these subjects needed hospitalization for asthmatic attack at least once. Table 1 presents the demographic, clinical and spirometric data of our subjects.

Genomic DNA extraction and hybridization technique

Blood was drawn for genomic DNA extraction from the 66 asthmatic subjects and 113 ethnically matched controls. Genomic DNA was prepared from 5 ml whole blood, using the Gentra System for DNA isolation. The gene encoding the β_2AR was examined for the two most frequent polymorphisms: Arg16Gly (A 46 → G) and Gln27Glu (C79 → G). For detection of A46 to G (Arg16Gly) we used the Amplification Refractory Mutation System [14], and for (C79 → G) (Gln27Glu) we used restriction site analysis. To detect A46 we used the following primers:

Forward: 5'CTTCTTGCTGGCACCCATTA3'.
Reverse: 5'CAATGACCAGATCAGCACAG3'.
To detect G46 we used the following primers:
Forward: 5'CTTCTTGCTGGCACCCATTG3'.
Reverse: 5'CAATGACCAGATCAGCACAG3'.
Amplification of exon 11 of HEXA gene was used as a control.

Polymerase chain reaction reaction consisted of 30 cycles: 94°C for 30 seconds, 58°C for 30 sec, and 72°C for 45 sec each. Final extension was at 72°C for 5 min. For detection of C79 to G (Gln27Glu) change, genomic DNA was amplified using the following primers:

Forward: 5' AGCCAGTGCGCTTACCTG3'.
Reverse: 5' AACTTGGCAATGGCTGTGAT3'.

The expected amplified fragment is 214 base pairs. PCR condition consisted of 30 cycles: 94°C for 30 sec, 59°C for 30 sec, and 72°C for 45 seconds each. Final extension was at 72°C for 3 minutes.

The C79 to G (Gln27Glu) eliminates restriction by BbVI, thus individuals without the change should exhibit the following fragments: 64, 56 and 94 bp. In a homozygous individual two fragments are expected: 64 and 150 bp, whereas in a heterozygous individual we expect to see four fragments: 150, 94, 64 and 56 bp.

The distribution of the allele in our non-asthmatic population was compared to three non-asthmatic groups described in the anglosaxic literature. Statistical evaluation was performed with the Fisher exact test. The study was approved by the Ethical Committee of Meir Hospital, Kfar Saba. A written informed consent was obtained from all subjects.

Results

Differences in β_2AR polymorphisms between our asthmatic subjects and their non-asthmatic controls

There was no significant difference between the frequency of the β_2AR polymorphisms in our asthmatic subjects and their non-asthmatic controls [Table 2].

Differences in β_2AR polymorphisms between various ethnic groups in Israel

Table 3 depicts the frequency of the allele in the control group. There were no significant differences between their distribution in Ashkenazi Jews, non-Ashkenazi Jews, or Arabs.

Differences in β_2AR polymorphisms between Israeli and western populations

Table 4 compares the frequency of the allele in our control group to three groups of Anglosaxic non-asthmatic subjects described in the

PCR = polymerase chain reaction

bp = base pairs

Table 2. Genotypes of our asthmatic subjects and controls

	NFA +NA +CSD	NFA + NA	NFA +CSD	NA	NFA	NA	CSD	All asthmatics	Non-asthmatic controls
No. of subjects	16 [24]	18 [27]	8 [12]*	6 [9]	11 [17]*	6 [9]	1 [2]	66 [100]	113**
Arg16	3 (19)	2 (11)	2 (25)	2 (33.3)	1 (9)	1 (17)	0 (0)	11 (17)	26 (23)
Gly16	6 (37)	5 (28)	2 (25)	2 (33.3)	1 (9)	1 (17)	0 (0)	17 (26)	35 (31)
Arg/Gly16	7 (44)	11 (61)	4 (50)	2 (33.3)	9 (82)	4 (66)	1 (100)	38 (57)	52 (46)
Gln27	8 (50)	11 (61)	5 (72)	2 (33.3)	4 (40)	3 (50)	0 (0)	33 (52)	53 (47.5)
Glu27	3 (19)	0 (0)	1 (14)	0 (0)	0 (0)	0 (0)	0 (0)	4 (6)	9 (8.5)
Gln/Glu27	5 (3)	7 (39)	1 (14)	4 (66.6)	6 (60)	3 (50)	1 (100)	27 (42)	49 (44)

* In one subject the genotype in locus 27 could not be determined. ** In two subjects the genotype in locus 27 could not be determined. Differences are not significant. [ ] = Percent of all subjects, ( ) = Percent of the group subjects.

Table 3. Frequency of ß$_2$AR allele in various Israeli ethnic groups

	Western Jews (Ashkenazi)	Non-Ashkenazi Jews	Arabs
No. of subjects	43*	41*	29
Gly16	0.5	0.52	0.62
Glu27	0.31	0.31	0.29

* In one subject the genotype in locus 27 could not be determined.

Table 4. Frequency of ß$_2$AR allele in non-asthmatic Israelis compared to three groups of non-asthmatic Anglo-Saxons

				Present study		
Author [Ref]	Liggett et al. [18]	Weir et al. [9]	Chong et al. [19]	Jews	Arabs	Total Israeli study population
No. of subjects	259	84	72	84	29	113
Gly16	0.59	0.61	0.55	0.51	0.62	0.54
Glu27	0.45[1]	0.4[2]	0.45[3]	0.31	0.29	0.30

[1] $P = 0.005$ versus Jews, $P = 0.001$ versus total Israeli population
[2] $P = 0.14$ (NS) = versus Jews, $P = 0.032$ in one-tailed test, 0.055 in two-tailed test versus total Israeli population
[3] $P = 0.027$ versus Jews, $P = 0.011$ versus total Israeli population

literature. There was no significant difference between our control group and the Anglosaxic groups in the frequency of Gly16. In contrast, Glu27 was significantly lower in our controls compared to two Anglosaxic non-asthmatic groups (P = 0.005 and 0.027, respectively, when considering only the Jews; and 0.001 and 0.01 when considering our whole population). This difference was borderline significant when comparing our total non-asthmatic population to the third group of Anglo-Saxons ($P = 0.032$ one-tailed and 0.055 two-tailed test).

Linkage between the alleles

All subjects who were analyzed in this study and were homozygous for Glu27 were homozygous for Gly16 as well. No one was heterozygous for codon 27 and homozygous for Arg16.

Discussion

We did not find significant differences between the frequency of either Glu27 or Gly16 in our asthmatic patients and their controls. There was also no significant difference in the frequency of the β_2AR polymorphisms between Jews of Ashkenazi origin, non-Ashkenazi Jews and Arabs. This is not surprising, since it has been proved that all Jews have a similar genetic pattern, related to that of the Middle East population [15].

On the other hand, the frequency of the allele in codon 27 but not in codon 16 in our non-asthmatic controls differs significantly from that observed for the anglosaxic population [Table 4] and was similar to that observed in a Turkish population [11], namely Glu27 of about 0.3 versus 0.4 in the anglosaxic population. In Afro-American and Chinese populations, the decrease in the frequency of Glu27 was more prominent than in our population, and the frequency of Gly16 also decreased [7,10].

The equality of distribution of Glu27 in our asthmatic subjects and their controls is apparently at divergence with *in vitro* studies, which indicated that Glu27 prevents the down-regulation of β_2AR [4], as well as with a publication indicating that it is positively related to decreased bronchial reactivity [16]. However, other investigators did not report any effects of polymorphisms in codon 27 on asthma severity [2,4,17]. This inconsistency with *in vitro* experiments was explained by linkage disequilibrium of Glu27 with Gly16, whose opposite effect (it enhanced down-regulation) is dominant [17]. This linkage disequilibrium was found also in our subjects.

The absence of significant difference between the distribution of Gly16 in asthmatic subjects and their controls or between the subgroups of the asthmatic subjects is at divergence with other publications from anglosaxic countries and the Far East [7,8], which found it to be increased in asthmatic patients who were admitted for asthmatic attacks [6], are corticosteroid-dependent [2], or have nocturnal asthma [5]. However, the western literature is contradictory on this point. Reishause et al. [2] found no correlation between Gly16 homozygosity and hospital admission, and Weir and colleagues [7] found no increase in the frequency of Gly16 in fatal and near fatal asthma.

In order to understand the contradiction between these

publications, we divided our subjects into subgroups according to the various attributes of severe asthma. Nevertheless, we could not find any significant correlation between them and β_2AR polymorphism. It is possible that in a much larger study population we could reach positive results. However, we believe that our data are sufficient to indicate that the impact of β_2AR polymorphism on asthma severity in our subjects is much less than in those investigated in the studies by Reishause et al. [2] or Turk et al. [5], where significant positive conclusions were drawn from results in a smaller population.

We conclude that the distribution of the allele in codon 16 and 27 of the β_2AR in our population was similar to that described for a Turkish population; namely, a decrease in the frequency of Glu27 as compared to Anglo-Saxons. We found no significant impact of the β_2AR polymorphisms on asthma severity. The cause for the various effect of this polymorphism in various geographic regions still remains to be determined, and it is possible that the balance between the various pathogenetic factors that determine the severity of asthma differs in various regions.

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