Association between CC16 Polymorphism and Bronchial Asthma

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Abstract: Background: Asthma is one of the most common chronic pediatric diseases, and is responsible for a significant proportion of school day losses. Asthma is influenced by genetic and environmental factors. The inheritance pattern of asthma demonstrates that it is a complex genetic disorder. Clara cell secretory protein (CC16) is an ideal candidate for involvement in an inherited predisposition to asthma owing to its chromosomal location, nature and function. Objective: to screen exons of CC16 gene on chromosome 11 for detection of sequence variation in families to determine whether these allelic polymorphism are associated with clinical asthma or not and to detect the relation between type of genetic polymorphism and asthma severity. Patients and Methods: This study included 20 stable asthmatic children with 21 of their atopic family members, also 11 healthy non atopic subjects were included as control group, all cases were subjected to genetic study methods including pedigree construction, PCR and detection of allelic polymorphism. Results: Significant correlation (90%) between homozygous CC16, 38 (AA) (75%) and 38 (AG) (15%) allelic polymorphism and increased prevalence of asthma was detected in families but there was no correlation between CC16 allelic polymorphism and asthma severity. Conclusion: There was a correlation between CC16 allelic polymorphism and increased prevalence of asthma and this gene is present also in normal subjects who are not triggered by environmental factors.
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Key words: CC16- Gene polymorphism – PCR- Asthma

1. Introduction:

Asthma is a chronic pulmonary disorder characterized by air way inflammation and bronchial hypereactivity (Hersberger et al., 2010). The etiology of asthma is complex. The dynamics that contribute to disease pathogenesis are multifactorial and involve overlapping molecular and cellular mechanisms, particularly the immune response to respiratory virus infection or allergen sensitization. Several factors may contribute to the development or exacerbation of asthma including age, host factors, genetic polymorphisms, and altered immune responses (Tauro et al., 2008).

Several genetic loci have been associated with asthma, and some of these associations have been replicated in independent studies. Several quantitative traits associated with asthma phenotype have been linked to markers on chromosome 11q13, the gene of Clara cell secretory protein (CC16) is an ideal candidate for involvement in an inherited predisposition to asthma owing to its chromosomal location, nature and function. CC16 gene is located on long arm of chromosome 11 within a region linked to atopy and increase airway responsiveness (Laing et al., 2000). The uniform increase of CC16 after swim exercise indicates that CC16 is of importance in epithelial stress, and may as such be an important pathogenic factor behind asthma development (Rombeg et al., 2010). High level of CC16 protein produced in the airway appears to function as an anti-inflammatory agents.

The assessment of respiratory risks in vulnerable population has thus for a long time relied on spirometric tests and self-reported symptoms which are relatively late and inaccurate indicators of lung damage. Blood tests measuring lung-specific proteins (pneumo proteins) such as Clara cell protein and surfactant – associated proteins are now available to evaluate the permeability and / or the cellular integrity of pulmonary epithelium (Bernard et al., 2004).

Aim of the study:

To screen exons of CC16 gene on chromosome 11 for detection of sequence variation in families to determine whether these allelic polymorphism are associated with clinical asthma or not and to detect the relation between type of genetic polymorphism and asthma severity.

2. Patients and Methods:

The present study included 20 stable asthmatic children during their regular follow up at Allergy Clinic, Pediatric Hospital, Cairo University. Their

ages ranged from 5-15 years. 21 subjects of their affected atopic family members were included in the study as well after a detailed questionnaire. Patients were classified according to National Asthma Education and Prevention Program (NAEPP) up dated (2002): Intermittent asthma: 9 cases; persistent asthma: Mild: 19 cases, Moderate 12 cases, Severe: 1 case. Another 11 healthy subjects with no personal or family history of asthma or other atopic manifestations were included as a control group. All cases were subjected to the following: A detailed history taking for asthma and other atopic manifestations using the standard sheet of allergy clinic, thorough clinical examination; peak expiratory flow rate (PEFR) for diagnosis of air way obstruction; routine laboratory investigations including CBC, urine and stool analysis; total serum IgE. by enzyme – linked immunosorbent assay (ELISA), and genetic study for all cases and control including pedigree construction and molecular study by PCR. Subjects were genotyped by restriction digestion of PCR products with Sau 961 endonuclease. The DNA was isolated from 3 ml whole blood using wizard genomic DNA purification Kit (Promega, Madison WI, USA). The sequence was obtained from published data (Hayward, 1995). These primers were supplied by Pharmacia . (Figures 1, 2, and 3)

Exon 1: 5 CAGTATCTTATGTAGAGCCC3`
5CCTGAGAGTTCCTAAGTCCAGG3`

Statistical methods:

All the above data were collected and analyzed using SPSS win statistical package version 11 by analysis of variance or students t-test. Correlations were studied by simple pearsons coefficient. Significance was defined as $P < 0.05$. Comparison between 3 groups was done using non-parametric ANOVA test.

3. Results:

The study included 20 stable asthmatic children and 21 subject of their affected atopic family members. Twenty one subjects (51%) were males and twenty subjects (49%) were females. Another 11 healthy subjects were included as control cases, 4 subjects (37%) were males and 7 subjects (63%) were females.

Table (1): shows comparison of selected data (age, sex, other atopic manifestations, asthma severity, absolute eosinophilic count (AEC), allelic detection of CC16 gene) in asthmatic group and control group. Table (2) shows comparison between selected data (sex, asthma severity, other atopic manifestations passive smoking, serum Ig E and inhaled corticosteroids) and types of allelic polymorphism either homozygous, heterozygous, or not detected genetic polymorphism. our results show that 31 cases were homozygous AA; 14 males (45.2%) and 17 females (54.8%), 6 cases were heterozygous AG; 5 males (83.3%) and 1 female (16.7%), while not detected allelic polymorphism GG in 4 cases; 2 males (50%) and 2 females (50%).

In homozygous allelic polymorphism (31 cases); 16 (51.6%) were atopic and 15 (48.4%) were non atopic, while heterozygous cases (6), 5 were atopic and 1 (16.7%) was non atopic and not detected allelic polymorphism in 4 cases all were atopic (100%). There was a history of passive smoking in 35 cases (85.3%) of them 27 cases were homozygous, 4 cases were heterozygous and 4 cases with not detected allelic polymorphism.

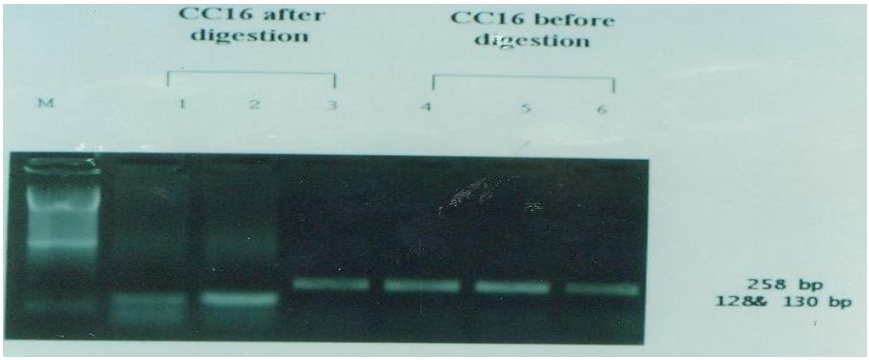

Figure 1. An agarose gel electrophoresis 2% stained with ethidium bromide showing three cases before and after digestion with restriction enzyme Sau 961.

M : Molecular DNA Marker (100 bp each)
Lanes 1,2 : 2 cases with homozygous alleles giving bands at 128 and 130 bp.
Lanes 3 : Case with undefined allele after digestion with bands at 258 bp.
Lanes 4-6 : Are the same 3 cases before digestion with bands at 258 bp.

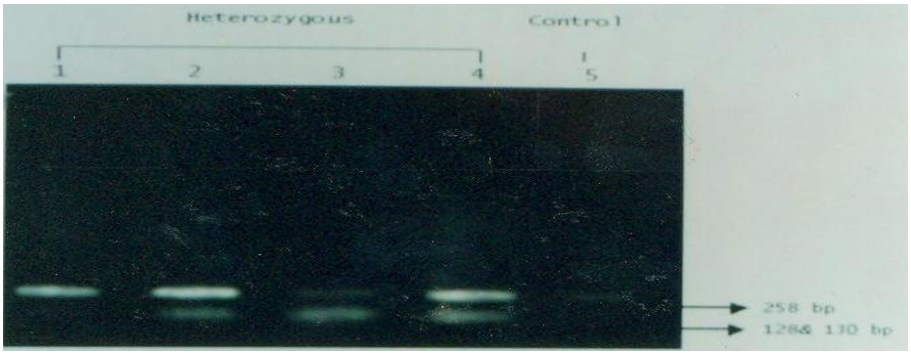

Figure 2. PCR products of CC16 gene after digestion with restriction enzyme Sau 961 showing cases with heterozygous alleles at 258 , 128, and 130 bp.
Lanes 1-4 : heterozygous alleles.
Lane 5 : control case.

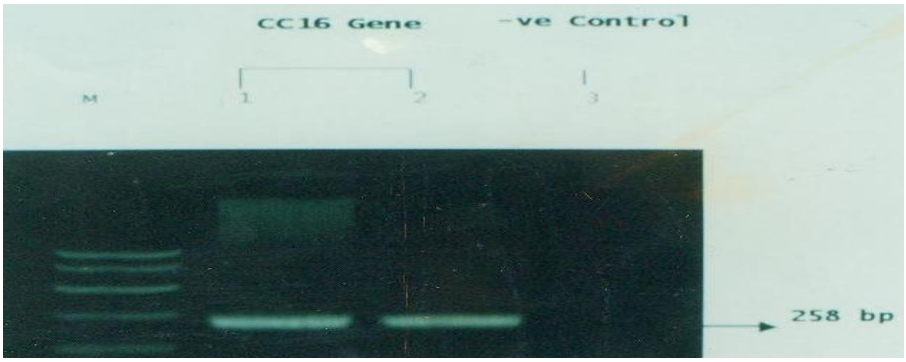

Figure 3. Agarose gel electrophoresis 2% stained with ethidium bromide showing strong gene expression of CC16 gene at 258 (Lanes 1,2) and negative control (Lane 3), M is PCR marker (1000, 750, 500, 300, 150, 50 bp).

Table 1. Characteristics of study and control groups .

Characteristics		Study group n=41	Control group N=11
Age (yr.)	Median ± SD Range	7 ± (13.86) (5-53)	6 ± (4.4) (4-35)
Sex	Male Female	21 (51.2%) 20 (48.8%)	4(36.4%) 7 (63.6%)
Other atopic manifestations	Atopic Non atopic	25 (61%) 16 (39%)	- 11 (100%)
* Asthma severity	* Intermittent * Persistent - Mild - Moderate - Severe	9 (22%) 19 (46.3%) 12 (29.3%) 1 (2.4%)	-
Serum IgE (IU/L)	Median ± SD Range	116.5 ± (179.14) (0-850.4)	81.7 (167.86) (17.40-508.2)
Absolute eosinophilic count (AEC) (cell/mm^3)	Median ± SD Range	385.35 ± (364.71) (.00 – 1470.00)	165.00 ± (184.41) (9-621.00)
Allelic Polymorphism	Homozygous Heterozygous Not detected	31 (75.6%) 6 (14.6%) 4 (9.8%)	1 (9.1%) - 10 (90.9%)

* Asthma severity based on guidelines of NAEPP (1997) update (2002).

Table 2. Comparison between selected data and types of allelic polymorphism

Genetic Group / Data	Homozygous (n=31)	Heterozygous (n=6)	Not detected gene (n=4)
Sex			
Male	14 (45.2%)	5 (83.3%)	2 (50%)
Female	17 (54.8%)	1 (16. 7%)	2 (50%)
Severity of asthma			
* Intermittent	5 (16.1%)	2(33.3%)	2 (50%)
* Persistent			
- Mild	15 (48.4%)	4 (66.6%)	-
- Moderate	11 (35%)	-	1 (25%)
- Severe	-	-	1 (25%)
Other atopic Mainfestations			
Atopic n= 25 (61%)	16 (51%)	5 (83%)	4 (100%)
Non atopic n= 16 (39%)	15 (48.4%)	1 (16.7%)	-
Passive smoking			
Yes (n=35)	27 (87.1%)	4 (66.7%)	4 (100%)
No (n=6)	4 (12.9%)	2 (33.3%)	-
IgE			
Median	118.6	26.3	96.8
Inhaled corticosteroids			
n= 13 (31.7%)	11 (35%)	-	2 (50%)

Table 3. Comparison between asthma severity and allelic polymorphism

Genetic Group / Asthma Severity	Homozygous (n = 31)	Heterozygous (n= 6)	Not detected (n=4)	Total (n=41)
Intermittent	5 (16.1%)	2 (33.3%)	2 (50%)	9 (22%)
Mild	15 (48.4%)	4 (66.6%)	-	19 (46.3%)
Moderate	11 (35.5%)	-	1 (25%)	12 (29.3%)
Severe	-	-	1 (25%)	1 (2.4%)

Based on guidelines provided by asthma education and prevention program, 1997 (NAEPP) (update to 2002).

Table 4. Comparison between serum IgE and allelic polymorphism

Genetic Groups / Serum IgE (IU/L)	Homozygous	Heterozygous	Not detected gene
Maximum	850.4	201.9	508.2
Minimum	0	0	17.4
Median	118.6	26.3	96.8
S.D.	± 19.74	± 76.59	± 153.83

Table 5. Comparison between AEC and asthma severity

Asthma severity / AEC	Intermittent	Mild	Moderate and Severe
Maximum	1470	990	1276
Minimum	27	0	20
Median	456.5	192	308.5
S.D.	± 505.4	± 284.4	± 3030.6

Table (3) shows comparison between asthma severity and allelic polymorphism, where no correlation could be found. Table (4) shows comparison between total serum Ig E and allelic polymorphism, where median serum IgE was higher in homozygous group followed by heterozygous group while in not detected allelic polymorphism median serum IgE was the least.

Table (5) shows statistical comparison between AEC and asthma severity, where AEC was higher in intermittent asthma followed by moderate and severe asthma and the least levels were those of mild asthma.

4. Discussion:

Asthma is a major health problem, it is the most common childhood disease and it is responsible for a significant proportion of school day loss. Asthma affects an estimated 5 to 15% of children in different populations and as such it is a major health care issue in most countries (Krowiec and Lemanske, 2004).

Because of the expression, pattern and function of CC16, it is an intriguing candidate gene for

chronic inflammatory lung diseases such as asthma. It is located on long arm of chromosome 11 q 13, a region occupied by several genes involved in the regulation of allergy and inflammation (Madsen et al., 2008).

In our work, genetic study was done to detect possible association between single nucleotide polymorphism (SNP) of CC16 gene exon 1 38 (AG) and asthma, and correlate between it and asthma severity. As regards asthma severity we followed NHLBI classification. The most common type of asthma was mild persistent asthma which represented 46.3% of cases, moderate persistent asthma represented 29.3%, intermittent asthma 22% and the least common was severe persistent asthma which represented 2.4%. The present work demonstrated that 53% came from urban area in comparison to 47% of patients came from rural areas. This is in accordance with the work of Reidler et al. (2001), who demonstrated that growing upon a farm confers protection against the development of atopy and asthma, also Von Mutius (2000), emphasized the importance of environmental factors in the pathogenesis, precipitation and aggravation of asthma and other allergic diseases. This could be explained by outdoor pollutants especially industrial smog that are responsible for the higher incidence of asthma in urban areas.

We found 61% of cases having other atopic manifestation which is consistent with Jane et al. (2000), who suggested a strong association between asthma and atopy. High incidence of smoking (85.4%) was found among the parents of our patients agreeing with the result of Norris et al. (2000), who showed higher incidence of parental smoking (62%). Also Shijubo et al. (1999), and Herman et al. (1998), suggested that tobacco smoking has been associated with 30% decrease in serum CC16 protein.

Atopy is a genetic susceptibility to produce IgE directed towards common environmental allergen. This laboratory finding together with elevated blood eosinophils with the presence of respiratory symptoms aid in the clinical diagnosis (Howard et al., 2000). AEC in our patients was significantly high (385.35±364 cells/cmm) than in control (165.09±184.4 cells/cmm) which is consistent with Micheal et al. (2005), who stated that eosinophilia greater than 4% or 300-400/ μ L supports the diagnosis of asthma but an absence of this finding is not exclusionary. High serum IgE level was detected in our cases compared with control which is consistent with the study of Micheal et al. (2005), they reported that total serum IgE levels greater than 100 IU are frequently observed in patients experiencing allergic reactions but it is not specific for asthma and may be observed in other allergic conditions.

PEFR is a useful measure of pulmonary function and is frequently utilized in asthma management, it also correlates with the degree of asthma severity (Van Helden et al., 2001) which is consistent with the present study.

We has observed a strong association between CC16 polymorphism and asthma. Thirty-one subjects (75%) of asthmatic patients were found to be homozygous for the gene 38 (AA), 6 subjects (15%) were heterozygous 38 (AG) and only 4 subjects (9.7%) were negative for genetic polymorphism 38 (GG), which were strongly different than control group where we found 10 cases (91%) negative for polymorphism and only one case (9%) was homozygous for polymorphism 38 (AA). This was consistent with Laing et al. (1998), who concluded significant relationship between genotype differences at position 38 of exon 1 of the CC16 gene and the risk of asthma which was increased 6.8 fold in homozygous subjects 38 (AA) and 4.2 fold in heterozygous 38 (AG). Also Ingrid et al. (2000), found that homozygous 38 (AA) subjects had reduced plasma levels of CC16 protein compared with 38 (AG) and (GG) subjects and stated that each additional 38 A allele was estimated to produce a 15% decrease in geometric means of plasma level of CC16 protein and associated with 63% increased risk of asthma.

Regarding control cases, one case was detected to be homozygous for polymorphism 38 (AA) which was consistent with Laing et al. (1998), who found 10.2% of participants homozygous for polymorphism 38 (AA). This may be due to lack of exposure to triggering factors of asthma. On the other hand, Mansur et al. (2002), observed no significant difference in the distribution of positive bronchial reactivity to metacholine across the three gene types.

Homozygous polymorphism 38 (AA) was detected in 48% of mild asthma cases, in 35% of moderate asthma cases and in 16.1% of intermittent asthma which is consistent with Sengler et al. (2003). Heterozygous polymorphism 38 (AG) was detected in 66.7% of mild asthma cases and in 33.3% of intermittent asthma. While not detected gene polymorphism was present in 25% of moderate asthma cases, 50% of intermittent asthma and in 25% of severe asthma cases. We could not correlate between the percentage of allele polymorphism and the severity of asthma due to small size of the sample.

Sengler et al. (2003), found that in asthmatic multicenter asthma study participant, FEV_1 value differ significantly between CC16 phenotypes, the lowest value were observed in children homozygous 38 (AA) followed by heterozygous 38 (AG). On the other hand Zhonghua et al. (2004), didn't reveal any

association between the genotype and allele frequencies of gene and severity of asthma. These contradictory results may be explained by race difference as it is possible that there is significant degree of genetic difference between populations.

5. Conclusion:

There is a correlation between CC16 allelic polymorphism and increased prevalence of asthma and this gene is present also in normal subjects who are not triggered by environmental factors. On the other hand no relation between CC16 allelic polymorphism and asthma severity could be detected in our study.

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