

Uteroglobin-related protein 1 gene –112G/A polymorphism and atopic asthma in Sicilian children

Luciana Rigoli, M.D.,* Chiara Di Bella, B.Sc.,* Vincenzo Procopio, B.Sc.,* Giuseppe Finocchiaro, M.D.#, Maria Amorini, B.Sc.,* Giuseppina Lo Giudice, B.Sc.,* Caterina Cuppari, M.D.,* and Carmelo Damiano Salpietro, Prof.*

ABSTRACT

The secretory protein, uteroglobin-related protein 1 (UGRP1), is expressed mainly in the lung and trachea and recently has been implicated in asthma. The –112G to A transition in the promoter was reported to be associated with asthma in the Japanese population. However, this has not been replicated in other studies. The aim of this study was to find the association of the UGRP1 gene polymorphism with atopic asthma in the Sicilian population. We conducted a transmission disequilibrium test (TDT) in 73 trios identified through 113 pediatric patients being treated for asthma. A case-control study also was performed by comparing the 113 unrelated asthmatic children and 230 unrelated healthy Italian subjects (121 children and 109 adults). The –112 G/A polymorphism was genotyped by the polymerase chain reaction–restriction fragment length polymorphism method and direct sequencing. The TDT revealed that the –112A allele was not preferentially transmitted from the parents to asthmatic offspring ($\chi^2 = 3.08$; $p = \text{NS}$). Neither the presence of at least one A allele in an individual's genotype (sum of the G/A and A/A genotype) nor the –112A allele was more prevalent among the asthma subjects than among the control subjects. Our results suggest that the –112G/A polymorphism does not play a significant role in the genetic predisposition of the UGRP1 gene in atopic asthma in the Sicilian population.

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Bronchial asthma is a complex, inheritable inflammatory disorder of the airways, characterized by reversible airflow obstruction, airway inflammation, and bronchial hyperresponsiveness.^{1–3} Recently, much work has been performed to find the responsible gene(s). Chromosomal region 5q31 harbors a number of genes associated with atopic phenotypes such as interleukin (IL)-4, IL-13, IL-5, and IL-9, the colony-stimulating factor 1 receptor gene (CSF-1R), and the β_2 -adrenergic receptor gene (ADRB2).^{4,5}

Recently, Niimi *et al.*⁶ found a new gene that encodes uteroglobin-related protein 1 (UGRP1), which is a secreted protein. It may function as a homodimer and it is similar to that of Clara cell secretory protein (CCSP/CC16), which is thought to function as an anti-inflam-

matory agent by inhibiting chemotaxis and phagocytosis of monocytes and neutrophils. Both exhibit decreased expression in inflamed mouse lungs. Therefore, it is possible that UGRP1 may play a role as an anti-inflammatory agent, such as CCSP/CC16, in the modulation of pulmonary inflammation.⁷

The UGRP1 gene is located on chromosome 5q31-q34 and is expressed in the lung and trachea. Recently, a G $\rightarrow$ A polymorphism was identified at –112 bp in the human UGRP1 gene promoter.⁷ The functional promoter polymorphism –112G $\rightarrow$ A in the UGRP1 gene was shown to be associated with bronchial asthma in a Japanese population,⁷ but these data have not been confirmed by other studies.^{8–10} The aim of this study was to find the association of the UGRP1 gene –112G/A polymorphism with atopic asthma in Sicilian children using a family-based and a case-control design.

MATERIALS AND METHODS

A group consisting of 113 trios (southern Italy) was recruited from among 113 (59 males and 54 females) children with allergies attending the Department of

From the *Genetics Unit, Department of Pediatrics, and #Department of Human Pathology, Medical School, University of Messina, Messina, Italy

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Address correspondence and reprint requests to Luciana Rigoli, M.D., Dipartimento di Scienze Pediatriche Mediche e Chirurgiche, Unità Operativa di Genetica ed Immunologia Pediatrica, Pad NI, Policlinico Universitario, 98125 Messina, Italy

E-mail address: luciana.rigoli@unime.it

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Pediatrics of the University of Messina (Sicily, Italy). Their mean age was 10.5 years (range, 6–14 years). We also selected 230 nonallergic unrelated controls who met all of the following criteria: (1) neither symptoms nor history of allergic disease such as asthma, atopic eczema, or allergic rhinitis; (2) no detectable dust-mite-specific IgE antibody; and (3) total serum IgE levels below the general age-adjusted population mean.

In this group, 121 children (59 boys and 62 girls) were studied and their median age was 11 years (range, 6–14 years). The median age of the remaining 109 adult controls (57 men and 52 women) was 40 years (range, 33–49 years). A full verbal and written explanation of the study was given to all family members interviewed, and all families gave informed consent and participated in the study.

Study Design

The study was performed from 2003 to 2006. We considered a diagnosis of asthma if any of the following signs or symptoms were present: (1) wheezing high-pitched whistling sound when breathing out; (2) history of cough, particularly worse at night; (3) recurrent wheeze, difficulty breathing, and chest tightness; (4) symptoms occurred or worsened at night, awakening the patient; (5) symptoms occurred or worsened in the presence of aerosol chemical, domestic dust mites, drugs, etc.; (6) reversible and variable airflow limitation was measured by using a spirometer (forced expiratory volume at 1 second and forced volume capacity) or a peak expiratory flow meter in children >5 years of age.

The subjects were stratified into intermittent, mild persistent, moderate persistent, and severe persistent groups based on combined assessments of symptoms and lung function. Asthmatic patients performed a pulmonary function test with a Multispiro PC pneumotachograph (Burke & Burke, Werzburg, Germany).

Atopy was defined by the presence of at least two of the following criteria:

1. A positive skin-prick test (wheal diameter ≥ 3 mm) to one or more common inhalant allergens.
2. Specific IgE of at least class 1 (>0.35 kU/L) to one or more inhalant allergens.
3. Elevated circulating total IgE: cutoff >100 kU/L.

We excluded patients with associated atopic dermatitis and allergic rhinitis.

Skin-Prick Test. Skin tests were performed on the forearm with an inhalant allergen panel from southern Italy: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Parietaria judaica*, *Phleum pratense*, and grass mix, *Artemisia*, olea, dog, cat, *Cladosporium*, and *Alternaria* (Soluprick; ALK, Horsholm, Denmark).

The measurement of the immediate-phase cutaneous responses was assessed 15 minutes later in accordance with recommendations of the European Academy of Allergology and Clinical Immunology.¹¹ Wheal size was expressed by the formula $(D + d)/2$, where D is the maximum diameter and d is the perpendicular diameter at the midpoint. A mean diameter of ≥ 3 mm was considered a positive result.

Total and Specific IgE Determination. Total IgE and specific IgE concentration in sera were determined with CAP system (Pharmacia and Upjohn Diagnostics, Uppsala, Sweden). Specific IgE concentration for inhalant allergen was detected according to the supplier's instructions.

Molecular Methods

Genomic DNA was extracted from whole blood.¹² Genotyping for the $-112G/A$ polymorphism was performed using a polymerase chain reaction (PCR) fragment amplified by the forward primer 5'-CTGTG-CAAGGGTTTATGCAA-3' and reverse primer 5'-CAAAGTGTGATGGCTGCTTTTGCAC-3'. PCR was performed in a final volume of 15 μ L containing 50 ng of genomic DNA, 2.5 mM of deoxyribonucleotides, 5.0 pM of each primer, 2 mM of $MgCl_2$, 1.5 μ L of Taq polymerase buffer, and 0.25 U of Amplitaq DNA Polymerase (Applied Biosystems, Foster City, CA). The PCR profile used was as follows: initial denaturation at 94°C for 5 minutes, followed by 30 cycles at 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 15 seconds. The final extension was at 72°C for 7 minutes. The $-112G/A$ polymorphism was identified by loss of the *HphI* restriction site (the G allele, 106-, 37-, and 43-bp fragments; the A allele, 143- and 43-bp fragments). The PCR products were digested with 2 U of *HphI* endonuclease (New England BioLabs, Inc., Beverly, MA) at 37°C, overnight. The samples were electrophoresed on a 3.0% agarose gel and visualized by ethidium bromide staining and ultraviolet transillumination. To confirm the accuracy of the genotyping, we sequenced 49 subjects randomly selected.

Statistical Analysis

Statistical analysis was performed through use of the STATA 9 program (StataCorp., College Station, TX).¹³ A transmission disequilibrium test (TDT) was used to analyze the pattern of allele transmission from heterozygous parents to all affected offspring calculating χ^2 and the corresponding p value.¹⁴ A value of $p < 0.05$ was considered statistically significant. Statistical significance was determined by χ^2 -test or Fisher's exact test for differences of allele and genotype frequencies.

Table 1 Genotype and allele distributions of the UGRP1 gene –112 G/A polymorphism in a case-control Sicilian population

	<i>n</i>	Genotype Counts (Frequencies)			<i>p</i> Value*	Allele Counts (Frequencies)		<i>p</i> Value*	HW χ^2 (<i>p</i>)
		G/G	G/A	A/A		G	A		
Asthmatic children	113	74 (0.65)	30 (0.27)	9 (0.08)	0.71	178 (0.80)	48 (0.20)	0.47	1.71 (NS)
Controls (children)	121	82 (0.68)	33 (0.27)	6 (0.05)		197 (0.81)	45 (0.19)		0.24 (NS)
Controls (adults)	109	71 (0.65)	34 (0.31)	4 (0.04)		176 (0.80)	42 (0.20)		0.04 (NS)

*Fisher's exact test was performed for G/G vs G/A and A/A.

HW = Hardy-Weinberg equilibrium.

RESULTS

Table 1 shows the results of case-control comparisons. The frequencies of children with asthma, children control subjects, and adult control subjects, with at least one A allele (G/A + A/A genotypes), were 34.5, 32.2, and 34.8%, respectively. The frequencies of the A allele in the asthmatic children, children control subjects, and adult control subjects were 21.2, 18.5, and 19.2%, respectively. In the three groups, the genotype distributions were no different from what was expected from the Hardy-Weinberg equilibrium for the UGRP1 gene –112 G/A polymorphism. The data of our case-control study did not show significant association between the –112G/A polymorphism and childhood asthma in the study population.

The TDT statistic and associated *p* value for all parents are shown in Table 2. We studied a total of 73 trios (both heterozygous parents and affected offspring). The TDT showed no preferential transmission of the A allele from the parents to asthmatic children ($\chi^2 = 3.08$; *p* = NS). Parental genotype distribution was in Hardy-Weinberg equilibrium for the –112G/A polymorphism.

DISCUSSION

In a case-control and a family-based design, we investigated the hypothesis that the human UGRP1 gene plays a role in the development of bronchial asthma in Sicilian children. We failed to replicate the association previously reported⁷ between the –112G/A polymorphism and asthma.

There are several possible explanations for the apparent inconsistency between our findings and the findings of Niimi *et al.*⁷ Indeed, the ethnic differences

may influence the results between the Italian and Japanese patients with atopic asthma.

The findings of our study were similar to data of other studies.^{8–10} In a white population, no association was found between the –112 G/A polymorphism of the UGRP1 gene and atopic asthma,¹⁰ as well in Indian⁸ and Japanese patients.⁹ Therefore, these findings suggest that the UGRP1 gene does not play a major role in the development of bronchial asthma.

The precise function of UGRP1 is unknown, but it is clear that UGRP1 is predominantly expressed in the lung¹⁵ and that it is a downstream target gene for the T/EBP/NKX2.12.1 homeodomain transcription factor.⁶ UGRP1 is a secreted protein that may function as a homodimer, as is the case with uteroglobin/CCSP, which is believed to modulate lung inflammatory response to infection. The amino acid similarities between UGRP1 and uteroglobin/CCSP family proteins are significant in the areas of signal peptide and anti-flammin.¹⁶ Recently, it has been shown that UGRP1 gene can suppress inflammation in the mouse model of allergic airway inflammation.^{17,18}

Furthermore, the UGRP1 gene was mapped in the area where many proinflammatory cytokine genes are located, including IL-3, IL-4, IL-5, IL-9, and IL-13, as well as genes encoding granulocyte macrophage CSF-2 and the receptor for CSF-1.^{17,18} Several studies indicate that the decreased expression of UGRP1 in allergic airway inflammation is mediated by IL-9 and IL-5.^{19,20} Therefore, UGRP1 has been proposed as a novel therapeutic candidate for treating lung inflammation in asthmatic patients.¹⁷

Table 2 Transmission of the UGRP1 gene –112 G/A polymorphism in Sicilian children with asthma of 73 trios

Allele	Variant Transmitted	Variant Not Transmitted	Total Transmission	Percentage	χ^2	<i>p</i>
–112A	29	44	73	39.7%	3.08	NS

To our knowledge, this is the first study in Sicilian children who have asthma. We found that the –112G/A allele frequency among 226 chromosomes from unrelated asthmatic children is almost the same as the frequency of our controls. In addition to the case-control comparison, we studied 73 trios by TDT. This family-based association test overcomes the problems of stratification, which can lead to both false positive and false negative findings. In our 73 trios, TDT analysis indicates that the –112G/A allele was not preferentially transmitted from the parents to asthmatic offspring, and this is consistent with our case-control association study results. From our results, we conclude that the –112G/A polymorphism of the UGRP1 gene could not exert a substantial influence on the inheritance of childhood asthma in our Sicilian population. However, it is possible that mutations in other, different genes can modulate UGRP1 gene expression in atopic asthma.

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