Alfa 1 Antitrypsin and Protease Levels in Puerto Rican Asthmatics: a Pilot Study

FEDERICO MONTEALEGRE, DVM, MS, Ph D*; ALEXIS DELGADO, RT*; ALIA TORO, MT*;
WANDA VARGAS, RN*; DOMINGO CHARDON, MD*; MANUEL BAYONA, MD, Ph D†‡
AND EDWARD CAMPBELL, MD**

A cross-sectional pilot study was conducted on a population of 119 asthmatics who had been recruited from the Emergency Room Department of a major hospital in Ponce, Puerto Rico. The purpose of the study was to determine the frequency of the MM, MS, and SS a-$_1$-antiprotease variants. Also, we analyzed the serum levels of the a-$_1$-antiprotease inhibitor, quantified the levels of serine proteases in homes of the asthmatic volunteers, and determined whether environmental levels of proteases, regardless of their sources, had any association with either asthma symptoms or a-$_1$-antiprotease inhibitor phenotypes. Our results do not support the role of the a-$_1$-antiprotease as a risk factor for asthma in the study population as previously reported. Patients who had visited the ED due to asthma on 3 or more occasions had significantly higher trypsin levels than those who had done so 2 or fewer times. Of those asthmatic patients who had daily symptoms, 40% had been exposed to high levels of elastase, and 33.3% to trypsin. Similarly, 52.9% of the patients with 2 or more hospitalizations a year had been exposed to high elastase levels, and 40.5% of asthma patients who had nocturnal asthma more than 3 times a week had been exposed to high levels of elastase.

Key words: Puerto Rican, Asthma, A1AT

National statistics show an increase in asthma prevalence; (1) it has been consistently shown that Puerto Ricans have the highest asthma prevalence than any other ethnic group in the United States (2,3,4,5). As a consequence, asthmatics seeking medial attention at Emergency Departments (ED) may impose a significative burden on the Health Care system. Published data shows that 26% of the asthmatics reported at least one hospitalization in a lifetime and 56% received medical attention in emergency room due to asthma exacerbations.[5] Although the causes for the high prevalence of asthma and high rates of ED visits are unknown, genetic factors may have a strong influence on asthma; at this time identifying these risk factors is difficult. Nevertheless, data suggest that polymorphisms in the $\beta2$ adrenergic receptor can influence the severity of asthma in Puerto Rican asthmatics (6,7,8). A second candidate gene is the CD14, which may be involved in triggering asthma, but only when the asthmatic is exposed to passive smoking.[9] Another possibility is the presence of $\alpha1$-proteasae (Alfa-1 antitrypsin) variants, which have been associated with Puerto Rican asthmatics who visit the ED (10). The latter molecule may be important in environmental exposures to exogenous proteases if there are quantitative or qualitative alterations in the anti-protease-protease balance.

Environmental risk factors also may have a strong association with the high prevalence of asthma and ED usage in Puerto Rican asthmatics. Changes in the life-style of the general population have lead many individuals to spend more than 95% of their time indoors, in insulated, temperature-controlled houses with low air exchange rates.[11] Given the conditions of the indoor environment, it is reasonable to assume that there would be an increase in the diversity and concentration of human exposure to airborne particles that can trigger asthma attacks. Compelling evidence from epidemiological studies in several parts of the world demonstrates that the development of asthma is promoted by indoor environmental irritants (IEI) (12,13). In particular, pro-inflammatory proteolytic allergens derived from domestic mites are now recognized to be an important cause of asthma sensitization, and clinical manifestations of asthma are related to the degree of indoor irritant exposure. Dust

*Department of Microbiology, Ponce School of Medicine; †Department of Epidemiology and Statistics, School of Public Health, University of North Texas;† and the **Department of Internal Medicine, University of Utah.†
‡Current Address: School of Public Health, University of Nevada.

Address correspondence to: Federico Montealegre, Department of Microbiology, Ponce School, of Medicine, P.O. Box 7004, Ponce, Puerto Rico 00732-7004, E mail: fmontealegre@psm.edu

mite allergen concentrations of 2 and 10 µg/g of bed dust have been proposed as exposure thresholds for the development of allergic sensitization and asthma symptoms, respectively (14,15,16,17). In addition to dust mites, exposures to cockroach allergens is a known risk factors for asthma; threshold levels for exposure to cockroach allergens are >=80 ng/g of dust (18). However, these epidemiological studies focused on determining dust mite or cockroach levels in specific geographic areas; (19) therefore, generalizations from their results may not be representative from many parts of the world.

A recent population-based study demonstrated that not all of the households sampled had high levels of allergens capable of triggering asthma symptoms (20). Data from the First National Survey of Lead and Allergies in Housing conducted in the United States found that 84% of US homes have detectable levels of dust mite allergens in sampled beds, but only 23% of the samples had high levels of mite allergens capable of producing asthma symptoms. In addition, the authors reported that cockroach allergen was detectable in the bedding in over 6% of the sampled homes. The authors concluded that the risk factors associated with these high allergen levels include the fact that the bedding being tested is located in: a single family homes, a low income households, a bedroom in which the humidity is high, an area where there is a musty and/or mildewy odor, a non-western parts of the US, an older homes (built before 1978), or a home whose inhabitants are non-Hispanic. However, the prevalence of asthma in these homes was not reported, therefore, it may be difficult to associate the reported levels with asthma symptoms. These results are highly informative and agree with findings in Puerto Rico. In past studies we have demonstrated that 33.3% of asthmatics are regularly exposed to indoor concentrations of dust mite allergens and 26.4% are exposed to cockroach (Bla g 1) allergens—both allergens are traditionally associated with asthma symptoms (21). In addition, our data showed that severe asthmatics are also exposed to low levels of dust mite and cockroach allergens, too low, in fact, to account for the severity of their condition. These findings point in favor of exogenous proteases (regardless of source) as environmental risk factors for asthma symptoms and severity in environments where dust mite and cockroach allergen levels are in low concentrations. In this context, the role of the antiproteases is important.

In an ED asthmatic population, the objective of the present pilot study was fourfold. First, to determine the frequency of the MM, MS, and SS α_1-antiprotease variants; second, to determine the serum levels of α_1-antiprotease inhibitor; third, to quantify the levels of serine proteases in homes of the asthmatic volunteers; and fourth, to determine whether environmental levels of proteases, regardless to their origin, had any association with asthma symptoms or with α_1-antiprotease inhibitor phenotypes.

Materials and Methods

Study volunteers and definitions. This was a cross-sectional, pilot study in which the study population was comprised of 119 asthmatic subjects recruited (consecutively) from the Emergency Departments of 2 major Hospitals associated with the Ponce School of Medicine Residency Programs in the City of Ponce, Puerto Rico. Patients were informed about the study, and those who were interested in participating as study volunteers were provided with our contact information. After signing the consent form (approved by the Institutional Review Board), each participant underwent a skin test and a blood draw; household dust was sampled; and a self-administered questionnaire was given. None of the subjects were related and participants represented different households. The diagnosis of asthma was made by an ED physician. A total of 105 subjects met the inclusion criteria, which included having or providing the following: 1) a specialist-based clinical diagnosis of asthma, 2) a blood sample (required for the determination of α_1-antiprotease inhibitor phenotype and levels), 3) a dust sample taken from his or her home, 4) a recent history of not taking medications such as antihistamines that could interfere with the skin prick tests, and 5) the completion of a self-applied questionnaire composed of 95 close-ended items on demographics, family history of allergy, and environmental tobacco exposures. Patients who were interested in participating underwent a skin prick test that was considered valid if the subject responded to the histamine positive control with a wheal greater than 3 mm but without a skin reaction to the negative control. A total of 14 (11.7 %) subjects did not meet the inclusion criteria. There were no significant differences in gender distribution or in the mean age between the subjects accepted for the study and those who were excluded (respectively, 36.7±2.0 and 38.9 ± 6.6, pairwise P=0.72). The study was initiated in June of 2003 and completed in May of 2005. This sample size (n=105) allowed for a 95% confidence in the detection of a minimum of 80% of the asthmatics with the MM phenotype and 11% of the asthmatics with the MS phenotype with 95% precision (22,23). (EpiInfo, CDC, Atlanta, GA). The sample size was calculated using previously published data for both phenotypes (24).

Asthma severity was analyzed as a nominal variable with two categories, severe or non-severe, based on any 4 of the 8 criteria included in the National Heart, Lung, and

Blood Institute guidelines (25). A severe case of asthma was defined as a patient with four of the following, in any combination: i) daily shortness of breath, ii) more than 3 visits to the emergency room due to asthma attacks in the 12 months prior to the interview; iii) more than 2 hospitalizations due to asthma attacks in the 12 months prior to the interview, iv) continuous symptoms between asthma attacks, v) marked limitation of physical activity, vi) nocturnal asthma symptoms more than 3 times a week, vii) poor attendance in school or work, and viii) emergency room time of more than 3 hours in a single visit.

Home visit questionnaire. A standardized, interviewer-administered, 242 item instrument was used to collect information about demographics, self-reported asthma symptoms, and risk factors. The questionnaire was standardized according to Kelsey *et al.* (26).

Dust mite allergen extracts for skin testing. Extracts from the domestic mites *Blomia tropicalis* and *Euroglyphus maynei* are not commercially available and were prepared as previously reported (27). A total of 1 gram of food-free *B. tropicalis* (Bt) and *Euroglyphus maynei* (Em) bodies were extracted in 20 ml of 0.2 M ammonium bicarbonate. The mixture was then placed in a beaker and sonicated for 15 minutes, on ice, at full output for 10 cycles, and placed in a refrigerated cabinet and incubated overnight under constant agitation. The extract was centrifuged at 6500 rpm for 1 hour and the resulting supernatant extensively dialyzed against water sterilized using a 0.22 µm pore size filter with a 3,000 kDa exclusion limit. After this, the extract was supplemented with 50% sterile glycerol. Mite cultures of *E. maynei* were kindly provided by Dr. B. Hart and extracts prepared as described.

Skin prick testing. Skin-prick tests were performed in the volar surface of the right arm by a trained nurse according to Pepys (28). Volunteers were tested using standardized commercial glycerinated extracts as previously reported (11). All of the patients were negative when skin tested with culture medium extract. The skin reaction was recorded at 15 minutes. The mean of the major diameter of the wheal and its perpendicular diameter were measured, and a skin reaction was considered positive if it had a wheal equal to or greater than 3 mm in the mean diameters. Positive (histamine 1 mg/ml) and negative (sterile PBS in 50% glycerol) controls, and an extract of the mite culture growth medium were included in the skin testing. For the prick tests, disposable acrylic copolymer needles were used (Morrow Brown, Oakhurst, NJ). All of the participants were tested using the same allergen lot and preparations. The following glycerinated extracts, were purchased from a single vendor (Hollister-Stier, Spokane, WA) and—with the exception of the dust mites *B. tropicalis* and *E. maynei* were tested on the study

population: house dust mites *Dermatophagoides pteronyssinus*, *D. Euroglyphus maynei*, *Blomia tropicalis* and *Euroglyphus maynei*, American cockroach *Periplaneta americana*, cat and dog dander. The general composition of the allergen mixes were as follows: Tree pollen 1:10 v/w: equal parts of Oleaceae *Fraxinus americana* (White Ash), Fagaceae *Fagus grandifolia* (American Beech), Betulaceae *Betula nigra* (River Birch), Juglandaceae *Juglans nigra* (Black Walnut), Rubiaceae *Populus deltoides* (Common Cottonwood), Ulmaceae *Ulmus americana* (American Elm), Juglandaceae *Carya ovata* (Shagbark Hickory), Aceraceae *Acer saccharum* (Hard Maple), Fagaceae *Quercus rubra (*Red Oak), Platanaceae *Platanus occidentalis* (American Sycamore), and Salicaceae *Salix nigra* (Marsh Black Willow). Weed: 1:10 w/v: equal parts of Asteraceae *Xanthium strumarium* (Cocklebur), Chenopodiaceae *Kochia scoparia* (Kochia), Chenopodiaceae *Chenopodium album* (Lamb's Quarters Marshelder), Chenopodiaceae *Monolepis pusilla* (Small Poverty-weed), Asteraceae *Iva ciliata* (True Marshelder), Compositae *Ambrosia trifida* and *Ambrosia elatior* (Ragweed mix), Amaranthaceae *Amaranthus retroflexus* (Rough Red Root), Chenopodiaceae *Salsola tragus* (Russian Thistle), Sagebrush Mix Asteraceae *Artemisia tridentata* (Common Sagebrush), *Artemisia ludoviciana* (Prairie Sage) and *Artemisia vulgaris* (Dark Mugwort), Dock Sorrel Mix Polygonaceae *Rumex acetosella* (Sheep Sorrel), Chenopodiaceae *Rumex hymenosepalus* (Curley dock) and Polygonaceae *Rumex crispus* (Yellow Dock), and Amaranthaceae *Amaranthus tuberculatus* (Western Waterhemp). Grass 1:10 w/v: equal parts of Poaceae *Poa pratensis* (Kentucky Bluegrass), *Dactylis glomerata* (Orchard Grass), *Agrostis alba* (Redtop), *Phleum pretense* (Timothy), *Anthoxanthum odoratum* (Sweet Vernal Grass), *Cynodon dactylon* (Bermuda Grass) and *Cynodon dactylon* (Johnson Grass). Mold Mix A: 1:10 w/v: equal parts of *Botrytis cinerea*, *Chaetomium indicum*, *Epicoccum nigrum*, *Fusarium vasinfectum*, *Geotrichum candidum*, *Helminthosporium interseminatum*, *Monilia sitophila*, *Mucor racemosus*, *Phoma herbarum*, *Penicillium mix*, *Pullularia pullulans*, *Rhizopus nigricans*, *Rhodotorula glutinis* and *Saccharomyces cerevisiae*. Mold mix B: 1: 10 w/v: equal parts of *Trichophyton schentieini*, *Cephalothecium roseum*, *Hormodendrum cladosporioides, Neurospora crassa*, *Streptomyces griseous*, *Scopulariopsis brevicaulis*, *Curvularia spicifera*, *Penicillium roseum*, *Mycogone* sp., *Nigrospora sphaerica*, *Paecilomyces variotii*, S*pondylocladium* sp., *Stemphylium botryosum*, *Trichoderma viride,* and *Trichosporon aquatile.* These allergen mixes have been used in previous studies conducted in Puerto Rico (29).

Dust Sampling and Processing. Dust samples from the entire mattress at the primary sleeping location of the test subject were collected using a vacuum cleaner (Medi-Vac® Products, Cheshire, England) at a rate of 2 min/m². Dust was placed in a plastic bag, which was in turn transferred to a refrigerated container. After being transported from the collection sites, dust was brought to the laboratory for weighing and storage (at -70°C), until extraction. Prior to extraction, the dust samples were sieved through a No. 45 mesh screen (355 :M). A total of 100 mg were mixed with 2 ml of PBS pH 7.4, without KCL, and supplemented with 0.05% Tween 80. The resulting combination was vortexed and then mixed on an orbital rotator (Scientific Industries, Inc, Bohemia, NY) within a refrigerated cabinet for 12 hours. The samples were centrifuged for 20 minutes at 2,500 rpm at 4°C. The resulting supernatant was removed, aliquoted in 100 Fl fractions, and stored at -80°C until analysis.

End-point measurement of Serine Protease Activity. Trypsin activity was detected in dust extracts by dissolving a total of 10 mg of the substrate benzoyl-DL-arginine p-nitroanilide (BapNA) in 2ml of DMSO. To this solution, a total of 18ml of 0.046M Tris containing 0.0115M of $CaCl_2$ buffer was added then a total of 100 Fl was placed in the wells of a 96-well microtiter plate. Next, 100 Fl of the dust extract was mixed and incubated at 37°C for 60 minutes, and analyzed on a Microtiter Plate Reader (Benchmark Plate Reader, Bio-Rad Laboratories, Inc., Hercules, CA) at OD 405 nm. The chymotrypsin assay was conducted under identical conditions as the trypsin assay but using the succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide substrate. Similarly, elastase was detected in dust extracts using succinyl-Ala-Ala-Pro-Leu-*p*-nitroanilide (SA_3-pNA). The reaction was allowed to proceed for 60 minutes, and the results were expressed in specific enzyme activity in nanomols of p-nitroanilide per minute/per gram of dust according to Exterkate (30). Results form these assays were expressed in EU/min/gram of dust. Since there are no established cutoff levels for these enzymes, we categorized the data in two levels, high and low, using the geometric mean (31).

Quantification and phenotyping of the a-₁-antiprotease inhibitor. The α-$_1$-antiprotease inhibitor concentration in serum from Puerto Rican asthmatics and identification of the phenotype of the α-$_1$-antiprotease inhibitor were s conducted at the α-$_1$-antiprotease (AAT) Inhibitor Deficiency Detection Center, Salt Lake City, Utah. The concentration of AAT was determined according to Silverman *et al.* (32). by an automated particle-concentration fluorescence immunoassay. Laboratory reference values for AAT are: a normal concentration for the MM phenotypes is 20-53 μM/L; for the MS phenotype, it is 18-52 μM/L; individuals with AAT deficiency have

concentrations less than 11 μM/L. Phenotyping was performed by isoelectric focusing of serum proteins in polyacrylamide gels, over a pH range of 4.2 to 4.9, essentially as described by Pierce and Eradio (33). The gels were stained with Coomassie Blue, and the results were compared with appropriate controls run simultaneously. Results were expressed in μM/L.

Statistical analysis. Stata version 7.0 (Stata Corporation, College Station, TX) was used to analyze the data. Frequencies were calculated for all of the categorical variables. For continuous variables, the mean, the geometric mean, the median, and the standard deviation were used for the descriptive analysis.

Results

Demographic characteristics. The study group was made up of unrelated Hispanics and included patients from all of the social strata. Overall, there were more female than male asthma patients (78.1% and 21.9%, respectively), and the female population was 8.7 years older than the male population; this difference was not significant (respectively, 38.6±20.1 and 29.9.± 19.9, pairwise P=0.06). Detailed information on the age distribution of the asthma patients is shown in Table 1. Six out 105 asthmatics were current smokers. Low frequency of home exposure to passive tobacco smoking was reported by 19 out of 101 (18.8%), while 33 out of 101 (32.7%) indicated high exposure to passive tobacco smoking.

Table 1. Age Distribution of the Study Population.

Age group (years)	Frequency	Cumulative frequency
<20	27	23.1
21-30	23	42.7
31-40	12	52.9
41-50	15	65.8
51-60	22	84.6
>60	18	100

Asthma characteristics. A total of 32 out of 105 (31.7%) patients were considered severe while 73 out 105 patients were classified as non-severe (68.3%). There were no differences between severe and non-severe asthma patients in their distribution by gender (31.4% and 82.5% females, respectively) or by age (respectively, 34.168±19.9 years and 37.82± 20.6 years, pairwise P=0.40). Severe asthmatics had a significantly higher number of ED visits than the non-severe (respectively, 11.8± 19.5 ED visits/year and 2.9 ± 4.0 ED visits/year, pairwise P=0.004), a significantly higher number of hospitalizations than the

non-severe asthmatics (respectively, 1.2±2.4 hospitalizations/year and 0.20 ± 0.5 hospitalizations/year, pairwise P=0.000), and spent more hours in the ED than the non-severe (respectively, 7.0 ± 3.5 hours/ ED visit and 5.2±2.6 hours/ED visit, pairwise P=0.005). The comparison of asthma characteristics between the severe and non-severe asthmatics is detailed in Table 2. Few asthmatics

Table 2. Summary of the Asthma Characteristics in the Study Population

| Characteristic | Asthmatics | | |
	Severe	Non-severe	P value
Daily frequency of exacerbations	18 (56.2%)	0 (0%)	0.000
Number of emergency room visits due to asthma in last 12 months	11.4 ± 18.5	2.8 ± 3.8	0.005
Number of hospitalizations per year	1.9 ± 2.4	0.2 ± 0.05	0.000
Continuos asthma symptoms between epidoses	27 (85.4%)	10 (13.7%)	0.000
Marked limitation pf physical activity	25 (78.1%)	12 (16.4%)	0.000
Frequency of nocturnal asthma > 3 times a week	21(67.7%	4 (5.5%)	0.000
Poor attendance at work or at school	12 (37.5%)	10 (13.7%)	0.006
Emergency Room time (mean hours±SD)	7.0 ± 3.5	5.2 ± 2.5	0.005

were active smokers, while the majority indicated that they were exposed to passive smoking (Table 3).

A1AT. Of the patients tested, 93 out of 105 were of the phenotype M, 12 out of 105 were of the phenotype MS. Within the MS phenotype, 11 out of 12 were heterozygous

Table 3. Phenotype And Smoking

| | Phenotypes | |
Smoking status	MM (n=93)	MS (n=12)
Current smoker		
Yes	4/93	2/12
No	89/93	10/12
Exposed to Passive Smoking		
No	45/93	4/12
Some	16/93	3/2
Frequent	28/93	5/93

for the S variant. In the M phenotype, 2 out of 93 expressed the M procida variant. All of the patients analyzed had normal levels of A1AT, and the blood levels were between 17.3 μM/L and 34.0 μM/L. We did not detect any significative associations between the M or MS phenotypes and asthma severity.

Protease levels. A total of 105 dust samples were analyzed for elastase and trypsin activity. Elastase and trypsin activity was detected in 87 out of 105 dust samples. The geometric mean for elastase was 0.41 (95% CI=0.31-0.55) EU/g dust, and for trypsin, the geometric mean was 0.39 (95% CI=0.29- 0.52) EU/g dust. Figure 1 illustrates the distribution of the trypsin and elastase levels in dust samples. We compared the levels of each enzyme (data not shown) and the asthma characteristics listed in Table 2II. Of these, asthmatics who had 3 or more ED visits due to asthma had significantly higher trypsin levels than their counterparts (respectively, 1.1±1.2 EU/g dust and 0.6±0.8 EU/g dust, pairwise p=0.04). We also analyzed the frequency of exposure to concentrations higher than 0.8 EU gr dust/minute. Forty percent of asthmatic patients who had daily symptoms were exposed to high levels of elastase; 33.3% to trypsin. Similarly, 52.9% of the patients with 2 or more hospitalizations a year were exposed to high elastase levels. Also, 40.5% of asthma patients who had nocturnal asthma more than 3 times a week were exposed to high levels of elastase. Details can be found in Table 4.

Skin reactivity. Of the 105 participants, 84 patients agreed to the skin test. A total of 74% of the asthmatics
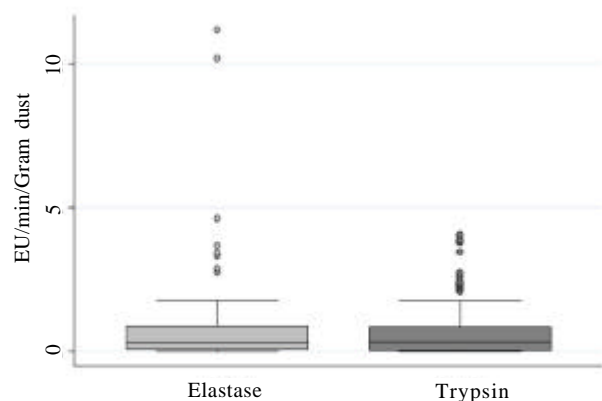
Figure 1. Boxplot of the Distribution of the Serine Proteases in Homes of ED Asthmatics

Table 4. Frequency of Exposure to Concentrations of Serine Protease and Clinical Outcomes

Variable/Protease	Relative frequency (%)
Daily shortness of breath	
Elastase (#0.31 EU/g dust)	55.5
Trypsin (#0.32 EU/g dust)	44.4
More than 3 ED visits due to asthma	
Elastase	45.9
Trypsin	40.5
More 2 hospitalizations due to asthma /year	
Elastase	58.8
Trypsin	64.7
Marked limitation of physical activity	
Elastase	48.6
Trypsin	54.0
Frequency of nocturnal asthma (> 3 times a week)	
Elastase	48.6
Trypsin	45.9
Poor attendance at work or school	
Elastase	52.0
Trypsin	48.0
Emergency room time (> 3 hours)	
Elastase	36.6
Trypsin	40.91

Table 5. Frequency of Positive Skin Reactions

Allergen	Frequency (n)
To al least one allergen	73.8% (62)
All mite species	69.0% (58)
Dermatophagoides pteronyssinus	60.7% (51)
D. farinae	58.3% (49)
Blomia tropicalis	58.3% (49)
Euroglyphus maynei	62.9% (52)
Mixed feathers	5.9% (5)
Mixed Shellfish	16.7% (14)
Rodents	14.3% (12)
Mouse epithelium	9.5% (8)
Rat epithelium	8.3% (7)
Pets	21.5%(18)
Cat hair and epithelium	17.9% (15)
Dog hair and dander	6.0% (5)
Pollen	17.9% (15)
Grasses	14.3% (12)
Trees	4,8% (4)
Molds	21.4% (18)
Alternaria alternata	4.8% (4)
Aspergillus fumigatus	1.2% (1)
Cockroach	38.1% (32)
Periplaneta americana	19.0% (16)
Blatella germanica	35.7% (30)

had at least 1 skin reaction, and were considered atopic. The most frequent skin reactivity was to dust mites (62 out of 84 - 58%), followed by cockroach (32 out of 85 - 38.1%) and molds (18 out of 84 -18%). Table 5 details the overall skin reactivity.

Discussion

The role of antiproteases in the airways is to protect cells from the damaging actions caused by endogenous and exogenous proteases. Quantitative and qualitative alterations in the balance between antiproteases-proteases can lead to asthma symptoms, and possibly severity. In their 1996 study, Shima and Adachi showed that decreased levels of the α-1 antiprotease is a risk factor for asthma.[34] However, contradictory results have been published by von Ehrenstein *et al.,* who's populational study showed that low levels of alpha1-AT in plasma do not enhance the risk of the development of asthma in children (35). In addition to its concentrations, the variants of α-1 antiprotease have been associated with asthma. Colp *et al* have suggested that in Puerto Rican asthmatics there is a positive correlation between the MS phenotype and asthma severity (36).

Our results do not support the association between α-1 antiprotease concentrations, its variants, and asthma

severity, as suggested by Colp *et al.* (33). In fact, the prevalence of the MS variant in our study population is 11.4% (Table #?) and is low in comparison to the one reported by Colp *et al.* (33). In their study, 18.1% of the Puerto Rican asthmatics expressed the MS variant. Our results are in agreement with those reported by Inselman *et al.,* who showed that the prevalence of the MS variant in Hispanic Caribbean asthmatic population is 11% (23). The contribution of the MS variant to asthma severity is highly variable; published data indicates that the prevalence of the MS variant in asthmatics varies from 1.2% in Poland (37) to 32% in Spain. The observed differences between our data and those of Colp *et al.,* exemplify the degree of difficulty in assessing the importance of variants. It is possible that our study population is more homogenous than the Puerto Rican population residing in the New York study. We also analyzed the frequency of the MM variant, which is the most prevalent. Our results are in agreement with those published by Colp *et al.* (33) and many others (34, 35,38,39). Of interest is the fact that we observed that 2 out of 93 asthmatics expressed the M procida variant. There are five known representatives of the null group of alpha 1AT-

deficiency alleles, which are genes incapable of producing alpha 1AT protein detectable in serum (40). The M procida variant reflects the asthmatic's probable Italian origin.[41] Currently, there are no data on the possible role of the M procida variant in asthma.

In most of the household dust samples in our study, we were able to measure serine protease levels that ranged from low to high.Our results indicate that a high percentage of asthmatics who had daily shortness of breath, more than 2 hospitalizations due to asthma in the last year, and nocturnal asthma symptoms more than 3 times a week were exposed to high levels of proteases. In fact, asthmatics who had 3 or more ED visits due to asthma had significantly higher trypsin levels than their counterparts. Overall, these results are in agreement with our previously published data, which demonstrate that—in Puerto Rico— exogenous serine proteases are associated with asthma (29). The importance of the our findings is that exogenous proteases can trigger asthma symptoms, by activating airway cells, causing them to produce inflammatory cytokines and bronchospasms. This is accomplished by activation of the Protease Activated Receptor (PAR), more specifically PAR-2 (42). PAR-2 is activated by serine proteases and is expressed in airway cells, including epithelial, smooth muscle, eosinophils, and others. In environmental exposures, the antiproteases—including α-1-antitprotease and secretory leukocyte protease inhibitor—may play an important role in neutralizing exogenous proteases. Therefore, the quantitative and qualitative integrity of these antiproteases becomes critical in protecting the airways. However, there are exogenous proteases such as the Der p 1,—an allergen produced by the dust mite *Dermatophagoides pteronyssinus,—*that will inactivate the α-1-antitprotease (43). Furthermore, proteases derived from *Aspergillus fumigatus,* a common environmental contaminant, are not inactivated by α-1-antitprotease (44). Our results suggest that exposures to exogenous serine proteases are not neutralized by the antiprotease system and are capable of inducing asthma symptoms.

In the present study, we classified asthma severity based on four out of eight of the recommended characteristics found in the National Asthma Education and Prevention Program Expert Panel Report 2: Guidelines for the Diagnosis and Management of Asthma; such classification was sufficient to identify differences in clinical characteristics among asthma patients (Table 2). Others have shown the difficulty of attempting to classify asthma severity based only on a reported history of symptoms (45). It is possible that the reliability of the classification in the present study was due to the fact that we used only two severity groups (severe and less-severe) as opposed to four severity groups (including patients with mild-intermittent and mild-persistent asthma). However, in our study, the key findings are: a) thirty-one percent of the asthmatics were considered severe, b) there were no differences between severe and non-severe asthma patients as to distribution by gender or by age, c) severe asthmatics had significantly higher numbers of ED visits, hours spent at the ED, and subsequent hospitalizations than their non-severe counterparts. Also, our results indicate that 73.8% of the patients were considered allergic, and the most common sensitizing allergens were those derived from dust mites, cockroaches, and pets. Although we did not measure the levels of dust mite, cockroach, and cat allergens present in the patient's home, the prevalence of positive skin reactions is indicative of environmental exposures to these allergens. These results are consistent with previously data (46).

Our study has limitations inherent to the study design, and other methodological issues. First, this is a pilot study and we used a small sample size. Therefore, our conclusions can not be extrapolated to other populations. Second, environmental measurements were made of serine proteases only; and other allergens, including those from dust mites, cockroaches, pets, and molds, were not measured. Therefore, our conclusions are suggestive rather than conclusive in nature. Third, we categorized the serine protease concentrations into two groups, low and high, based upon the geometric mean; there are no published cutoff levels for these analytes. This means that the distributions of concentrations within each category may be overestimated. However, these categorizations are a starting point that will lead into an appropriate methodology for establishing clinically relevant serine protease threshold levels. In spite of these limitations, our study has provided useful data in regards to the role of the α-1-antitprotease in Puerto Rican asthmatics.

In conclusion, our results suggest that the α-1-antitprotease may not be involved in asthma symptoms or severity in the study population, and exogenous proteases may play an important role in triggering asthma. Future studies with a larger sample size are required to elucidate the role of the exogenous proteases on asthma symptoms or asthma severity.

Resumen

Un estudio transversal se realizó en una población de 119 asmáticos que fueron reclutados de la Sala de Emergencia de un hospital en Ponce. El objetivo del estudio fue determinar la frecuencia de los variantes MM, MS y SS de la α-$_1$-antiproteasa. También se analizaron los niveles

séricos de la α-$_1$-antiproteasa y se determinó si los niveles ambientales de las proteasas de serina en las residencias de los asmáticos, irrespectivamente de su origen, tenían alguna asociación con los síntomas del asma, y los variantes de la α-$_1$-antiproteasa. Nuestros resultados no apoyan el papel de la α-$_1$-antiproteasa en el asma en la población del estudio como previamente sugerido. Pacientes con mas de 3 visitas a salas de emergencia tenían niveles significativamente más elevados de tripsina que aquellos pacientes con 2 o menos visitas. El 40% y el 33.3% de los asmáticos con síntomas de asma diarios estaban expuestos a altos niveles de elastasa y de tripsina, respectivamente. Al igual, 52.9% de los pacientes con 2 o mas hospitalizaciones por año estaban expuestos a altos niveles de elastasa. El 40.5% de los pacientes que sufrían de síntomas nocturnos de asma mas de 3 veces por semana estaban expuestos a altos niveles de elastasa.

Acknowledgments

References

1. Vogel G. New clues to asthma therapies. Science. 199;276:1643-1646.
2. CDC. Self-reported asthma prevalence among adults-United States, 2000. MMWR.2001;50:682-686
3. Homa DM, Mannino DM, Lara M.Asthma mortality in U.S. Hispanics of Mexican, Puerto Rican, and Cuban heritage, 1990 1995. Am J Respir Crit Care Med. 2000 Feb;161(2 Pt 1):504 9.
4. Perez-Perdomo R, Perez-Cardona C, Disdier-Flores O, Cintron Y. Prevalence and correlates of asthma in the Puerto Rican population: Behavioral Risk Factor Surveillance System, 2000. J Asthma. 2003;40(5):465-74.
5. Montealegre F, Bayona M. An estimate of the prevalence, severity and seasonality of asthma in visitors to a Ponce shopping center. P R Health Sci J. 1996 Jun;15(2):113-7.
6. Salari K, Choudhry S, Tang H, Naqvi M, Lind D, Avila PC, Coyle NE, Ung N, Nazario S, Casal J, Torres-Palacios A, Clark S, Phong A, Gomez I, Matallana H, Perez-Stable EJ, Shriver MD, Kwok PY, Sheppard D, Rodriguez-Cintron W, Risch NJ, Burchard EG, Ziv E. Genetic admixture and asthma-related phenotypes in Mexican American and Puerto Rican asthmatics. Genet Epidemiol. 2005 Jul;29(1):76-86.
7. Choudhry S, Ung N, Avila PC, Ziv E, Nazario S, Casal J, Torres A, Gorman JD, Salari K, Rodriguez-Santana JR, Toscano M, Sylvia JS, Alioto M, Castro RA, Salazar M, Gomez I, Fagan JK, Salas J, Clark S, Lilly C, Matallana H, Selman M, Chapela R, Sheppard D, Weiss ST, Ford JG, Boushey HA, Drazen JM, Rodriguez-Cintron W, Silverman EK, Burchard EG. Pharmacogenetic differences in response to albuterol between

8. Burchard EG, Avila PC, Nazario S, Casal J, Torres A, Rodriguez-Santana JR, Toscano M, Sylvia JS, Alioto M, Salazar M, Gomez I, Fagan JK, Salas J, Lilly C, Matallana H, Ziv E, Castro R, Selman M, Chapela R, Sheppard D, Weiss ST, Ford JG, Boushey HA, Rodriguez-Cintron W, Drazen JM, Silverman EK; Genetics of Asthma in Latino Americans (GALA) Study. Lower bronchodilator responsiveness in Puerto Rican than in Mexican subjects with asthma. Am J Respir Crit Care Med. 2004 Feb 1;169(3):386-92.
9. Choudhry S, Avila PC, Nazario S, Ung N, Kho J, Rodriguez-Santana JR, Casal J, Tsai HJ, Torres A, Ziv E, Toscano M, Sylvia JS, Alioto M, Salazar M, Gomez I, Fagan JK, Salas J, Lilly C, Matallana H, Castro RA, Selman M, Weiss ST, Ford JG, Drazen JM, Rodriguez-Cintron W, Chapela R, Silverman EK, Burchard EG. CD14 Tobacco Gene-Environment Interaction Modifies Asthma Severity and Immunoglobulin E Levels in Latinos with Asthma. Am J Respir Crit Care Med. 2005 Jul 15;172(2):173-82.
10. Colp C, Talavera W, Goldman D, Green J, Multz A, Lieberman J. Profile of bronchospastic disease in Puerto Rican patients in New York City. A possible relationship to alpha 1-antitrypsin variants. Arch Intern Med. 1990 Nov;150(11):2349-54.
11. Fernandez-Caldas E, Fox R. Environmental control of indoor air pollution. Med Clin North America. 1992;76:935-951.
12. Platts-Mills TAE, Thomas WR, et al. Dust mite allergens and asthma: report of a second international workshop. J Allergy Clin Immunol. 1992;89:1046-1060.
13. Weiss KB, Gergen PJ, Crain CF. Inner city in asthma: The epidemiology of an emerging US public health concern. Chest. 1992;101:362-367.
14. Kuehr J, Frischer T, Meinert R, Barth R, Forster J, Schraub S, et al. Mite allergen exposure is a risk for the incidence of specific sensitization. J Allergy Clin Immunol. 1994;94:44-52.
15. Sporik R, Holgate ST, Platts-Mills TA, Cogswell JJ. Exposure to house-dust mite allergen (Der p I) and the development of asthma in childhood. A prospective study. N Engl J Med. 1990;323:502-507.
16. Platts-Mills TA, Ward GW Jr, Sporik R, Gelber LE, Chapman MD, Heymann PW. Epidemiology of the relationship between exposure to indoor allergens and asthma. Int Arch Allergy Appl Immunol. 1991;94:339-345.
17. Platts-Mills TA, Hayden ML, Chapman MD, Wilkins SR. Seasonal variation in dust mite and grass-pollen allergens in dust from the houses of patients with asthma. J Allergy Clin Immunol. 1987;79:781-791.
18. Arruda LK, Vailes LD, Mann BJ, et al. Molecular cloning of a major cockroach (Blatella germanica) allergen, Bla g 2. J. Biol. Chem. 1995;270: 19563-19568
19. Platts-Mills TA, Vervloet D, Thomas WR, Aalberse RC, Chapman MD. Indoor allergens and asthma: report of the Third International Workshop. J Allergy Clin Immunol. 1997;100:S2-S24.
20. Arbes SJ Jr, Cohn RD, Yin M, Muilenberg ML, Burge HA, Friedman W, Zeldin DC. House dust mite allergen in US beds: results from the First National Survey of Lead and Allergens in Housing. J Allergy Clin Immunol. 2003;111(2):408-414.
21. Montealegre F, Fernandez B, Delgado A, Fernandez L, Roman A, Chardon D, Rodriguez-Santana J, Medina V, Zavala D, Bayona M. Exposure levels of asthmatic children to allergens, endotoxins, and serine proteases in a tropical environment. J Asthma. 2004 Jun;41(4):485-496.
22. Dean AG, Dean JA, Coulobier D, Brenkel KA, Smith DC, Burton

AH, Dicker RC, Sullivan K, Fagan RF, Arner TG. Epi Info, Version 6: a Word Processing, Database, and Statistics Program for Public Health on IBM-compatible Microcomputers. Centers for Disease Control and Prevention, Atlanta, Georgia, USA, 1995.

23. Wayne W. Daniel, Biostatistics: a foundation for analysis in the Health Sciences, 5th edition, John Wiley & Sons, 1987., p.157.

24. Inselman LS, Bognacki NS, Evans HE. Alpha1-antitrypsin phenotypes in asthmatic children of Hispanic background. Chest. 1978 Nov;74(5):600.

25. National Heart, Lung and Blood Institute. National Asthma Education Program Expert Panel Report. Guidelines for the Diagnosis and Management of Asthma, 1997.

26. Kelsey J, Thompson W. Douglas, Evans Alfred (eds). Methods in Observational epidemiology. Oxford Press, 1986. New York, Oxford, pp 222.

27. Montealegre F, Quinones C, Torres N, Goth K. Detection of serine proteases in extracts of the domestic mite Blomia tropicalis. Exp Appl Acarol. 2002;26(1-2):87-100.

28. Pepys J. Skin testing. Br J Hosp Med.1975;14:412-417.

29. Montealegre F, Quinones C, Michelen V, Bayona M, Fernandez-Caldas E, Vazques O, Colon F, Chardon D, Garcia M. Prevalence of skin reactions to aeroallergens in asthmatics of Puerto Rico. P R Health Sci J. 1997;16(4):359-367.

30. Exterkate, FA. Differences in short peptide-substrate cleavage by two-cell-envelope-located serine proteinases of Lactococcus lactis subs. cremoris are related to secondary binding specificity. Appl Microbiol Biotechnol. 1990 Jul;33(4):401-406.

31. Montealegre F, Fernandez B, Delgado A, Fernandez L, Roman A, Chardon D, Rodriguez-Santana J, Medina V, Zavala D, Bayona M. Exposure levels of asthmatic children to allergens, endotoxins, and serine proteases in a tropical environment. J Asthma.2004;41:485-496.

32. Silverman EK, Miletich JP, Pierce JA, Sherman LA, Endicott SK, Borze GJ, Campbell EJ. Alpha 1-antitrypsin deficiency: High prevalence in the St. Louis area determined by direct population screening. Am Rev Respir Dis. 1989;140:961-966.

33. Pierce JA. Eradio BG. Improved identification of antitrypsin phenotypes through isoelectric focusing with dithioerythritol. J Lab Clin Med. 1979;94:826-831.

34. Shima M, Adachi M. Association of respiratory symptoms with serum protease inhibitors and albumin levels in Japanese children. Int J Epidemiol. 1996 Dec;25(6):1213-1219.

35. von Ehrenstein OS, Maier EM, Weiland SK, Carr D, Hirsch T, Nicolai T, Roscher AA, von Mutius E. Alpha1 antitrypsin and the prevalence and severity of asthma. Arch Dis Child. 2004 Mar;89(3):230-231.

36. Colp C, Pappas J, Moran D, Lieberman J. Variants of alpha 1-antitrypsin in Puerto Rican children with asthma. Chest. 1993 Mar;103(3):812-815.

37. Szczeklik A, Torowaska B, Czerniawska-Mysk G, Opolska B, Nizankowska E. Serum alpha–1-antitrypsin in Bronchial asthma. Am Rev Respir Dis. 1974;109;487-489.

38. Katz RM, Lieberman J, Siegel SC. Alpha-1 antitrypsin levels and prevalence of Pi variant phenotypes in asthmatic children. J Allergy Clin Immunol. 1976;57:41-45.39. Brocard M, Mauriat M, Lacour B, et al. Phénotypes de l'alpha-1-antitrypsine et asthme de l'enfant. Rev Fr Allergol. 1982;22:25-28.

40. Takahashi H, Crystal RG. Alpha 1-antitrypsin Null (isola di procida): an alpha 1-antitrypsin deficiency allele caused by deletion of all alpha 1-antitrypsin coding exons. Am J Hum Genet. 1990 Sep;47(3):403-413.

41. Luisetti M, Seersholm N. á 1-Antitrypsin deficiency á 1: Epidemiology of á 1-antitrypsin deficiency. Thorax. 2004;59(2):164-169.

42. Reed CE, Kita H. The role of protease activation of inflammation in allergic respiratory diseases. J Allergy Clin Immunol. 2004;114:997-1008.

43. Kalsheker NA, Deam S, Chambers L, Sreedharan S, Brocklehurst K, Lomas DA.. The house dust mite allergen Der p1 catalytically inactivates alpha 1-antitrypsin by specific reactive centre loop cleavage: a mechanism that promotes airway inflammation and asthma. Biochem Biophys Res Commun. 1996;221:59-61.

44. Robinson BW, Venaille TJ, Mendis AH, McAleer R, Allergens as proteases: an Aspergillus fumigatus proteinase directly induces human epithelial cell detachment. J Allergy Clin Immunol. 1990;86:726-731.

45. Ford JG, Meyer IH, Sternfels P, Findley SE, McLean DE, Fagan JK, Richardson L. Patterns and predictors of asthma-related emergency department use in Harlem. Chest. 2001;120:1129-1135.

46. Montealegre F, Quinones C, Michelen V, Bayona M, Fernandez-Caldas E, Vazques O, Colon F, Chardon , Garcia M. R. Prevalence of skin reactions to aeroallergens in asthmatics of Puerto Rico. P R Health Sci J. 1997;16:359-367.