

Estimates of the Prevalence
and Number of Fibromyalgia Syndrome Patients
and Their Alpha-1 Antitrypsin Phenotypic Distribution
in Ten Countries

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ABSTRACT. Objectives: During the last few years, clinical, epidemiological, and pathological evidence has suggested that inherited alpha-1 antitrypsin [AAT] deficiency might play a role in the

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development of the fibromyalgia syndrome [FMS], probably because of the loss of AAT anti-inflammatory efficacy. The objective of this study was to estimate the prevalence and number of FMS patients, and their AAT phenotypic distribution worldwide. Methods: A critical review selecting reliable studies on the subject.

Results: Studies on AAT gene frequencies and FMS prevalence were retrieved for ten countries worldwide, namely Canada, the United States of America [USA], Denmark, Finland, Germany, Italy, the Netherlands, Spain, Sweden, and Pakistan. The severe deficiency Z allele was found in all these countries, with very high frequencies in Denmark and Sweden [23 and 27 per 1,000, respectively], high frequencies in Italy and Spain [16 and 17], intermediate frequencies in Germany, the Netherlands, Canada, and the USA [10 to 14], and a low frequency in Pakistan [nine per 1,000]. The calculated prevalence of AAT deficiency and the number of FMS patients with AAT deficiency were 1/10 and 25,408 in Canada, 1/11 and 478,681 in the US, 1/9 and 3,124 in Denmark, 1/36 and 726 in Finland, 1/16 and 48,523 in Germany, 1/13 and 84,876 in Italy, 1/15 and 9,639 in the Netherlands, 1/4 and 114,359 in Spain, 1/11 and 9,065 in Sweden, and 1/25 and 85,965 in Pakistan. Our calculations predict that AAT deficiency would remain undetected in around nine percent of FMS patients, with about eight percent of them carrying moderate deficiency phenotypes [MS, SS, and MZ], and less than one percent with severe deficiency phenotypes [SZ and ZZ].

Conclusions: Therefore, AAT phenotype characterization should be recommended in FMS patients and the possible efficacy of AAT replacement therapy in severe deficiency FMS patients should warrant further studies. doi:10.1300/J094v15n04_03 [Article copies available for a fee from The Haworth Document Delivery Service: 1-800-HAWORTH. E-mail address: <docdelivery@haworthpress.com> Website: <<http://www.HaworthPress.com>> © 2007 by The Haworth Press, Inc. All rights reserved.]

KEYWORDS. Fibromyalgia syndrome, fibromyalgia syndrome prevalence, alpha-1 antitrypsin deficiency, epidemiological studies

INTRODUCTION

Alpha-1 antitrypsin [AAT] is the prevalent serine protease inhibitor in humans permeating most body tissues and acting as a broad-spectrum anti-inflammatory molecule (1-6). Under normal conditions, the liver secretes about 34 mg/kg of AAT every 24 hours, but this quantity may increase up to fourfold or more in response to inflammatory, infectious, and tumorous processes, as well as during pregnancy (7). Hepatocytes are the primary source of AAT [over 70 percent of AAT is secreted by the liver], but other cells, including monocytes, macrophages, lung and intestinal epithelial cells, endothelial cells, dendritic cells, natural killer cells, lymphoblasts, bone marrow, pancreas, kidney, adenocarcinoma cells, and possibly other tissues are believed to synthesize supplementary quantities of this protein.

The AAT gene is located on the long arm of the 14th chromosome, being inherited as an autosomal codominant trait. The most common version [allele], called M, produces normal levels of AAT. Most people have two copies of the M allele [MM], one from each parent. Two al-

tered versions producing moderately low or very low levels of AAT are called S and Z, respectively. Individuals with two copies of an altered allele [ZZ or SZ] develop severe AAT deficiency.

One form of AAT deficiency is a genetic disorder affecting all major racial subgroups, especially Caucasians (8). Using the technique of isoelectric focusing, about 100 genetic variants of AAT proteinase inhibitor [PI] phenotypes have been identified to date. The alphabetical designation of these variants is based on their mobility in an electrophoretic field at alkaline pH. Rapidly migrating variants are designated by the earlier letters of the alphabet [A to M], and those migrating more slowly by the latter ones, with Z variant being the slowest. In clinical practice, phenotypes usually found are MM [normal phenotype expressing around 100 percent, range 80 to 120 percent of AAT], MS [slightly deficient, expresses around 70 to 80 percent of AAT], SS [moderately deficient, it expresses around 55 to 65 percent of AAT], MZ [moderately deficient, expresses around 50 to 60 percent of AAT], SZ [moderately-severely deficient, expresses around 30 to 40 percent of AAT], and ZZ [severely deficient, expresses

around 10 to 20 percent of AAT; see Figure 1]. Variants with an increased risk of developing some disease are those in which deficient or null alleles are combined in homozygous or heterozygous states encoding AAT plasma concentrations below 60 percent. Most pathologies related to AAT deficiency are associated with the Z allele and, in clinical practice, 95 percent of patients have a ZZ phenotype. The remaining five percent belongs to SZ, MZ, and other highly rare deficient or null phenotypes (3,7,9-11).

A systematic review of current literature carried out by the writing group of the AAT Deficiency Task Force provides clear scientific evidence of the relationship between AAT deficiency and chronic obstructive pulmonary disease, liver cirrhosis, and necrotizing panniculitis. The available grade of evidence is moderate for the relationship between AAT deficiency and bronchial asthma, bronchiectasis, and systemic vasculitis. For AAT deficiency and several other diseases occasionally reported, the available level of evidence is weak (11).

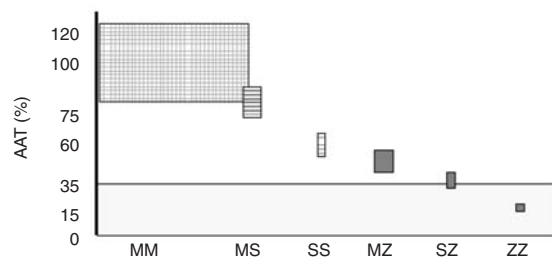
The fibromyalgia syndrome [FMS] is an idiopathic, chronic pain syndrome defined by widespread musculoskeletal pain and generalized tender points. The worldwide prevalence of this complex syndrome ranges from 0.5 to five percent (12,13). The FMS primarily affects women in their 30s to 60s at approximately a 9:1 to 20:1 female to male ratio. Both FMS eti-

ology and pathogenesis are still unknown. For the moment there is no available curative treatment for this condition (12-15).

A possible relationship between Z-AAT deficiency and FMS has been recently reported (16-19), on the basis of: 1. a clinical alert, consisting in the repetitive positive response of FMS symptoms to AAT replacement therapy in two ZZ FMS patients (16), 2. the finding of abnormal muscle and endothelium deposits of AAT in the muscle biopsy of an FMS patient with AAT-MZ deficiency (17) [Figures 2 and 3], 3. an epidemiological study (18) carried out in Asturias [Northern Spain] between 2003 and 2005, showing that severe deficiency allele PI*Z had been found in a twofold higher frequency in severe FMS patients than in general population [Table 1], 4. a series of muscle biopsies in 23 severe FMS patients (17) showing AAT deposits in both the lumen and the endothelium of muscle small vessels and myofibrils, as well as different degrees of muscle atrophy in FMS patients with AAT deficiency [Table 2]. These abnormal findings were much more remarkable in FMS patients with Z alleles [according to gradient ZZ>SZ>MZ>MS]. Interestingly, AAT tissue deposits were neither in a polymeric, nor in a protease cleaved, C-terminal form, suggesting that in AAT deficiency-related FMS AAT accumulations in both muscles and vessels might represent other molecular forms of AAT, such as native, latent, and/or complexed with other proteins.

The objective of the present study is to estimate the prevalence and the number of FMS patients and their AAT phenotypic distribution worldwide through the analysis of available studies and the later selection of those with reliable results on AAT.

FIGURE 1. Serum Levels of Alpha-1 Antitrypsin According to Phenotypes. Graphical presentation of most frequent PI phenotypes in various countries, namely the United States, Canada, and central Europe. Both bar size and width are proportional to each phenotype percentage. Thirty-five percent indicates the minimum protection level for lung tissues.



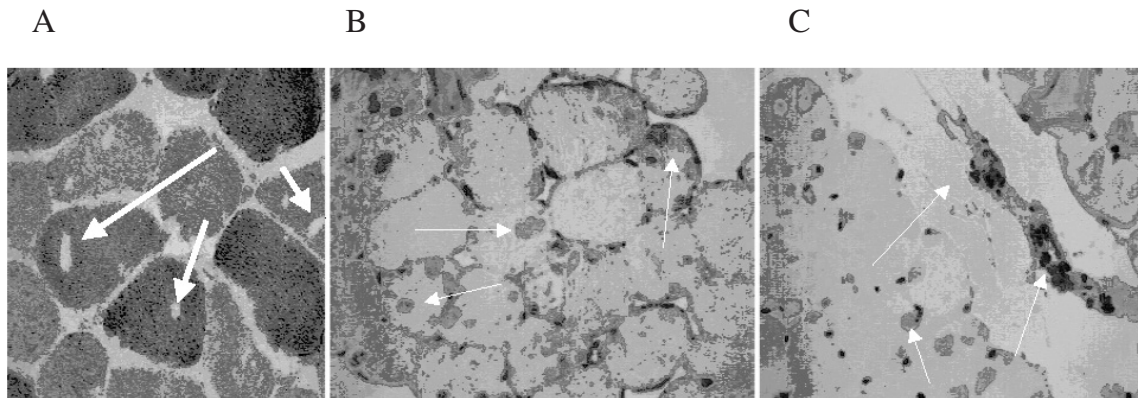
AAT = alpha-1 antitrypsin

MATERIALS AND METHODS

Sources of Fibromyalgia Syndrome and Alpha-1 Antitrypsin Data on Both the Prevalence and the Gene Frequency Used in the Present Study

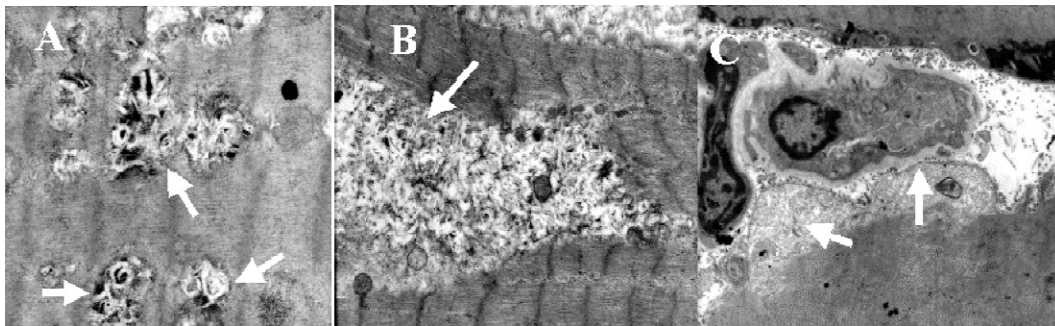
The present study employs available data from epidemiological studies performed by other authors to determine the prevalence of

FIGURE 2. Muscle Biopsy of an MZ Patient with Fibromyalgia Syndrome



A: NADH \times 40: Scattered internal and subsarcolemmal non-rimmed vacuoles [arrows].
 B: AAT \times 40: Positive staining of vacuoles with rabbit anti-human Alpha1-Antitrypsin [arrows].
 C: AAT \times 40: Alpha-1 Antitrypsin in endothelial smooth muscle layer of arteriole and subsarcolemma [arrows].

FIGURE 3. Muscle Biopsy of an MZ with Fibromyalgia Syndrome Using an Electron Microscope



A: Myofibrillar deposits of amorphous material.
 B: Impressive muscle aggregates interrupting sacromere structure.
 C: Subsarcolemmal deposits close to the lumen of a small vessel.

FMS in worldwide general population (20-42). Papers were obtained through a variety of sources, including searches of peer-reviewed literature in PubMed and Web of Science, in the Library of the National Institutes of Health, and of papers published from 1965 up to June 2006. Additional relevant studies were obtained by searching in the bibliography of the first articles retrieved. Some articles published in local journals of difficult availability were also obtained after their request to their authors. Selected studies for the present analysis fulfilled the following criteria: 1. FMS classification and diagnosis performed through the application of the 1990 American College of Rheumatology

[ACR] classification criteria (12), 2. samples representative of the general population of a whole country, 3. in absence of studies on the total population from a whole country, prevalence reported for well designed studies on subjects representative of the general population of a specific region and results extrapolated to the population of the whole country was employed. Over the past several years, the authors of the present manuscript have worked on AAT deficiency epidemiology, publishing their research in different peer-reviewed journals (43-47). The authors' personal databases were combined to generate a common database used in this analysis. For the present study, they em-

TABLE 1. Comparison of PI*S and PI*Z Deficiency Allele Frequencies [per 1,000] and the Calculated Prevalences of Five Deficiency Allelic Combinations [MS, MZ, SS, SZ, and ZZ] in the General Population and in Fibromyalgia Syndrome Patients from the Central Region of the Asturias Province in Northern Spain (ref. 18)

Sample composition [sample size]	Total Alleles N	Alleles type N			Mean gene frequency expressed in $\times 1,000$ [95% CI]		Hardy-Weinberg calculated prevalence [1/x]					
		M	S	Z	PI*S	PI*Z	MM	MS	MZ	SS	SZ	ZZ
General population [1,111 subjects]	2,223	1,956	223	44	100 [88-113]	19.7 [14-27]	1.29	6	29	100	254	2,573
FMS [250 patients]	500	448	32	20	64 [45-90]	40 [25-62]	1.25	9	14	244	195	625

Odds Ratio Z 2.06 [1.16-3.63] P = 0.007, significant difference
Odds Ratio S 0.93 [0.62-1.41] P = 0.73, no significant difference

FMS = fibromyalgia syndrome patients, CI = confidence interval

TABLE 2. Summary of Muscle Biopsy Findings in 23 FMS Patients [13 With and 10 Without Inherited Alpha-1 Antitrypsin Deficiency], Compared to Control Samples

FMS Patients				
AAT phenotype	AAT deposits in small vessels	Myofibrillar vacuoles	Myofibrillar AAT Deposits	Type 2 B muscle fibers atrophy
MM [10]	7 [70%]	0	0	0
MS [5]	5 [100%]	2 [40%]	2 [40%]	2 [40%]
MZ [3]	3 [100%]	2 [66%]	2 [66%]	
SZ [3]	3 [100%]	3 [100%]	3 [100%]	2 [66%]
ZZ [2]	2 [100%]	2 [100%]	2 [100%]	2 [100%]
Controls				
	Endothelial AAT deposits	Subsarcolemmal vacuoles	Subsarcolemmal AAT deposits	Others
Muscle biopsy of 5 normal [MM] subjects	5 [100%]	0	0	
Muscle biopsy of 2 polymyositis MM patients	2 [100%]	0	0	Severe inflammatory data
Muscle biopsy from 1 COPD ZZ no-FM patient	1 [100%]	0	0	0
Liver biopsy samples from 3 known ZZ-cirrhotic patients	-	-	-	AAT liver aggregates ATZ11 positive [100%]

FMS = fibromyalgia syndrome, AAT = alpha-1 antitrypsin

Staining methods: haematoxylin-eosin, Gomori trichrome, ATPase [pH: 4.3, 4.6, 4.3], esterase, NADH, succinate dehydrogenase, cytochrome oxidase, phosphorylase, oil red, desmine, polyclonal-AAT [rabbit anti-human alpha-1-antitrypsin], ubiquitin, three antipolymeric AAT monoclonal antibody ATZ11 and anti-AAT carboxy-terminal peptide monoclonal antibody.

ployed available data from epidemiological studies performed by other authors to determine the frequencies of deficiency allele combinations for PI*S and PI*Z in healthy control cohorts of individual case studies of European, American, Australian and New Zealand, African, and Asian populations. Data from these individual cohorts for a given country were combined to obtain mean frequencies for PI*M, PI*S, and PI*Z alleles. The allele frequencies were subsequently used to calculate the total

number of individuals in each of the five major defective phenotypic classes of interest [namely MS, MZ, SS, SZ, and ZZ] in the total population of each of these countries.

Formulas to develop estimates of the allele frequency of gene prevalence, the number of deficiency allele combinations, and 95 percent confidence intervals [95% CI] were discussed in earlier papers (43-47). Allele frequencies were expressed as the total number of PI*S and PI*Z, whether in homo- or heterozygotes, per 1,000 alleles of all PI types.

The prevalence of every phenotype was calculated by applying Hardy-Weinberg statistics, where $p^2 + 2pq + q^2 = 1$ (48).

Data on the number of individuals in every country and other important population details, such as male/female ratio, population age structure, ethnic group composition, and percentages were obtained from the Central Intelligence Agency World Factbook database, updated in 2006 (49).

To assess the statistical reliability of each survey, we calculated the coefficient of variation [*cv*] for PI*S and PI*Z frequencies in each control cohort. The *cv* provides an estimate of the precision [or better, the imprecision] of the results of each survey. Imprecision measures data dispersion with respect to the mean and depends on the total number of studied alleles and gene frequencies of PI*S and PI*Z found. Precision is inversely proportional to the values of *cv*; therefore, the smaller the value of *cv*, the greater precision. The 95% CI of gene frequencies for a given control cohort are larger when gene frequencies are smaller. Thus, reliable studies depend on the number of subjects in each control cohort, as well as on gene frequencies of PI*S and PI*Z. Numerical Precision Factor Scores [PFS] to assess the statistical quality in terms of precision [or imprecision] of each selected survey were generated as follows:

$$\text{for both S and Z } \left[\begin{array}{l} Zcv = \frac{100 \times (Zul - Zll)}{4 \times Zfr} \text{ and} \\ Scv = \frac{100 \times (Sul - Sll)}{4 \times Sfr} \end{array} \right], \text{ the mean } cv \text{ value}$$

$$\overline{cv} \text{ was calculated through the formula } \overline{cv} = \frac{Zcv + Scv}{2} \text{ and the numerical PFS, through}$$

the formula $PFS = 500 \times \frac{1}{cv}$ [where Sul = S 95%CI calculated upper limit; Sll = S 95% CI calculated lower limit; Zul = Z 95%CI calculated upper limit; Sfr = S frequency; Zll = Z 95%CI calculated lower limit; Zfr = Z frequency, and 500 is a factor to get a value scale for PFS from 0 to 12].

Microsoft Excel spreadsheets were developed to record and process data from individual genetic epidemiological studies to determine PI subtypes in control populations in each country. To ensure accuracy, all calculations were made through embedded spreadsheet

formulas for all the estimates of prevalence and number of carriers, and deficiency allele combinations for PI*S and PI*Z with 95% CIs, as described in earlier papers (8,43-47).

Reliable selected studies on AAT gene frequency and prevalence used for the present analysis fulfilled the following criteria: 1. AAT phenotype performed by isoelectrofocusing or antigen-antibody crossed electrophoresis, 2. statistical precision factor score of ≥ 5 for South-Western Europe, ≥ 4 for Central and Northern Europe, Northern America, and Australia and New Zealand, and ≥ 3 for Eastern European, African, and Asian countries, and 3. samples representative of the general population.

RESULTS

We only found ten countries worldwide with reliable studies on both FMS prevalence and AAT gene frequency (50-91). These studies were performed in Canada, the United States [US], Denmark, Finland, Germany, Italy, the Netherlands, Spain, Sweden, and Pakistan. Unfortunately, other countries with reliable studies on FMS prevalence (39-42), namely Bangladesh [FMS prevalence 3.3 to 4.4 percent], Mexico [FMS prevalence 1.4 percent], Brazil [FMS prevalence 2.5 percent], and Turkey [FMS prevalence 3.6 percent] could not be included in this analysis as they do not contain any survey on AAT gene frequency.

Details of Selected Studies

Canada: Eight control cohorts [two Eskimos [Igloodik] and Metis [Saskatchewan] and five Caucasians] were selected from studies on AAT gene prevalence performed in Canada (50-57). Precision Factor Score mean was 6.4 for all Canada. Weighted mean frequency for the PI*S allele [per 1,000] was 39.0 [35.0-42.8] and for PI*Z was 13 [11-15].

Four studies on FMS prevalence were retrieved from Canada. According to the selection criteria used in the present study, the selected study was that of McNally et al. (20). This study describes a self-reported epidemiology, clinically diagnosed, of FMS in Canada, using data collected from the Canadian Com-

munity Health Survey, Cycle 1.1, 2000 (20). The study provides with a mean FMS prevalence rate of 1.1 percent [0.33 for men and 1.83 for women] in the general population between 15 to 65 years, with a female-to-male ratio of 6:1. Samples were obtained from the general population of Canadian Atlantic provinces, Quebec, Ontario, Prairie Provinces, and British Columbia. Others small-scale studies carried out in Ontario were rejected since their results might be less reliable should they be extrapolated to the national level.

United States: A total of 29 control cohorts [two Asian Americans, six African Americans, three Hispanics, two Mexican Americans, and 16 Caucasians] were selected from studies on AAT gene frequency, performed in the United States of America (58-65). Precision Factor Score mean was 7.3. For Asian Americans, the mean frequency for PI*S allele [per 1,000] was 0.0 [0.0-0.3] and the mean gene frequency for PI*Z was 0.0 [0.0-0.3]. For African Americans, the mean frequency for PI*S allele was 11.1 [7.4-16.4] and the mean gene frequency for PI*Z was 2.6 [1.0-5.9]. For Hispanics, the mean frequency for PI*S allele was 50.3 [35.4-70.7] and the mean gene frequency for PI*Z was 9.1 [3.7-20.8]. For Mexican Americans, the mean frequency for PI*S allele was 42.1 [25.5-67.8] and the mean gene frequency for PI*Z was 0 [0-9]. For Caucasians, the mean frequency for PI*S allele was 31.6 [29.9-33.3] and the mean gene frequency for PI*Z was 14.9 [13.8-16.2]. For the whole USA, the weighted mean frequency for PI*S allele was 30.8 [28.8-32.9] and the mean gene frequency for PI*Z was 14 [13-16].

To estimate FMS prevalence in US population, the results of an age- and sex-specific study performed in the general population of Wichita, Kansas by Wolfe et al. (24) was used. The Wichita population is approximately 88 percent Caucasian and, although FMS prevalence in this population might not reflect the real prevalence in US population as a whole, this local study was used for the present analysis due to the lack of general population studies in the US. Reported FMS prevalence in age 18 and older was two percent [95 percent CI: 1.4-2.7], with a lower prevalence in men [0.5 percent] than in women [3.4 percent].

Denmark: A total of two control cohorts were selected from studies performed in Copenhagen (66,67). Precision Factor Score mean was 12. The mean frequency for S allele [per 1,000] was 28 [26-30] and the mean gene frequency for Z was 27 [25-29].

For FMS prevalence, the Prescott et al. study (26) was selected, based on a national health interview survey carried out by the Danish Institute for Clinical Epidemiology on approximately 6,000 randomly selected Danish citizens. For this study 1,219 subjects from Eastern Denmark were asked about widespread muscle pain. A clinical examination was performed on 65 persons who answered positively to the questionnaire. Eight subjects, all female, met the ACR criteria (12) for FMS, resulting in a calculated prevalence of 0.66 percent [0.28-1.29].

Finland: A total of four control cohorts were selected from studies performed in Finland (68-71), one from Lapps, or the Sami people, and three from Caucasians. The calculated statistical value of the PFS was 4.3. The weighted mean frequency for PI*S was 7 [5-10] and mean gene frequency for PI*Z was 6.6 [4.5-9.7].

For FMS prevalence, the Makela and Heliovaara study (27) was selected. This cross-sectional study was performed on 8,000 Finns aged 30 years or older, invited for a screening and a main examination of musculoskeletal disorders. Estimated FMS prevalence was of 0.75 percent, twice as prevalent in women as in men.

Germany: A total of eight control cohorts were selected from studies performed in Germany (72-76). Combined PFS of these cohorts was 5.1. The calculated mean gene frequency [per 1000] was 21 [18-24] for PI*S and 10 [8-12] for PI*Z.

Due to the lack of reliable studies on the whole country, the Schochat and Raspe study (29) was selected on FMS prevalence. This study population-based and cross-sectional study was performed on 3,174 females, aged 35 to 74 years, residing in Bad Sackningen [southern Germany]. A stratified random sample of 653 German women was clinically examined, 36 of them fulfilling ACR criteria of FMS (12), resulting in a mean calculated prevalence of 1.13 percent.

Italy: Eight cohorts were collected from Italy (77-82). Mean PFS was 7.6. PI*S and mean gene frequencies for PI*Z were of 23 [21-25] and 16 [15-18] per 1,000.

Data on FMS prevalence were obtained from MAPPING study (30), where questionnaires were sent to a random sample of 3,664 individuals aged 18 and over, stratified by age and gender, selected from the practice list of 16 general practices. Trained rheumatologists carried out structured visits where subjects were asked about musculoskeletal symptoms. The FMS prevalence was 2.2 percent [1.36-3.19].

The Netherlands: A total of five control cohorts were selected from studies performed in The Netherlands (83-85). Combined PFS these five studies was 4.8. Calculated mean gene frequency [per 1,000] was 22 [28-26] for PI*S and 11 [8-14] for PI*Z.

The Picavet et al. study (33) was selected for FMS prevalence. This study is a prospective cohort study of a random sample in the general Dutch population aged 25 years and older. Mean FMS prevalence was of 1.15 percent [0.2 percent in males and 2.1 percent in females].

Spain: Four control cohorts were selected from studies performed in Spain (86-89). Combined PFS for these four studies was 7.7. Calculated mean gene frequency [per 1,000] was 104 [96-113] for PI*S and 17 [14-21] for PI*Z.

Results of EPISER study (35) on 2,998 subjects aged 20 years and older were used for the present analysis to calculate FMS prevalence. Subjects were randomly selected by stratified multistage cluster sampling from the census of 20 municipalities. Trained rheumatologists carried out structured visits and underwent a standardized physical examination. Mean FMS prevalence was 2.4 percent [1.5-3.2], with the gender distribution of 0.2 percent males and 4.2 percent females.

The recent epidemiological study on FMS and AAT deficiency carried out in Asturias [Spain] (18), evidencing that PI*Z allele had a twice higher frequency in FMS patients than in the general population [Table 1], was rejected for the present analysis because these FMS patients suffered severe FMS, and supposing that AAT deficiency could be related to severe FMS, this fact could result in the aggregation of Z genes.

Sweden: A control cohort was selected from studies performed in Sweden (90). The calculated statistical value of mean PFS for the study on Swedish Caucasians was 8.8. Gene frequency for PI*S allele was 24 [18-32] and 23 [17-30] for PI*Z per 1,000.

For FMS prevalence, the present analysis selected the Lindell et al. study, carried out in a general population from southwestern Sweden. The estimated FMS prevalence was 1.3 percent [0.8-1.7].

Pakistan: A control cohort of 269 healthy unrelated people from Karachi was selected to calculate the AAT gene frequency in Pakistan (90). The calculated statistical value of mean PFS was 2.5. Gene frequency for PI*S was 11 [4.5-29] and 9 [3.4-23] for PI*Z.

A study on FMS prevalence carried out in three localities representative of the social spectrum of northern Pakistan reported an FMS prevalence of 2.1 percent, with a male-to-female ratio of 1:13. This study compared rheumatic symptoms amongst 1,997 adults. There were 295 positive respondents; of 274 evaluated by a rheumatologist, according to the ACR criteria (12), 42 cases were diagnosed with FMS. The survey instrument employed was the core questionnaire of the Community Oriented Program for Control of Rheumatic Diseases, started jointly by the World Health Organization and the International League Against Rheumatism.

Estimation of the Total Number of Fibromyalgia Syndrome Patients in the Selected Countries

To estimate the population at risk for FMS in each of these countries, we employed the following variables: the total population of each country; the number of subjects included in the range of ages selected for each study, the male-to-female ratio of FMS prevalence reported, and the estimated weighted FMS prevalence. Therefore, the calculated population with FMS in each of these ten countries would be as follows:

Canada: Total population: 33,098,932, estimated population at risk [males and females between 15 and 65 years]: 69 percent, FMS weighted mean prevalence re-

ported: 1.1 percent. Calculation: $33,098,932 \times 69/100 \times 1.1/100 = 251,221$ FMS patients.

United States: $298,444,215 \times 89.7/100 \times 2/100 = 5,354,089$ FMS patients

Denmark: $5,450,661 \times 81.3/100 \times 0.66/100 = 29,247$ FMS patients

Finland: $5,231,372 \times 66.7/100 \times 0.75/100 = 26,170$ FMS patients

Germany: $82,422,229 \times 85.8/100 \times 1.13/100 = 799,116$ FMS patients

Italy: $58,133,509 \times 86.3/100 \times 2.2/100 = 1,103,723$ FMS patients

The Netherlands: $16,491,461 \times 77.1/100 \times 2.3/200 = 146,222$ FMS patients

Spain: $40,341,462 \times 51.8/100 \times 2.4/100 = 501,525$ FMS patients

Sweden: $9,016,596 \times 83.3/100 \times 1.3/100 = 97,000$ FMS patients

Pakistan: $165,803,560 \times 61/100 \times 2.1/100 = 2,123,944$ FMS patients

The results of the total numbers estimates of FMS patients in each of these ten countries and their PI-phenotypic distribution are summarized in Table 3.

DISCUSSION

Our tabulation demonstrates that both PI*S and PI*Z alleles are found in all FMS populations of the countries studied, and that there exists very striking differences for the distribution of PI*S and PI*Z alleles among these countries. Of the 10 selected countries, the lowest FMS prevalence was found in Denmark and Finland [< 1 percent], followed by Canada, Sweden and the Netherlands [about one percent], the US [two percent], and Italy, Spain and Pakistan [2 to 2.4 percent].

Since the calculated numbers are a reflection of both the specific PI*Z and PI*S frequency

and the total population of each country, the largest number of ZZ individuals was found in the USA, followed by Italy and Germany, with the remaining countries having a lesser number. SZ phenotypes are relatively abundant in the USA and Spain, with a lesser number in the remaining countries.

We are aware that these data should be considered as an approximation, since our calculations might have bias related to the quality of the studies selected for analysis and to the sources of the subjects recruited. It is important to note the remarkable lack of epidemiological studies in some extensive geographic regions of several countries, as well as the existence of marked differences in the contribution of AAT and FMS data among the different regions of the same country. The unbalanced contribution of the different regions of a given country should be taken into account for some countries of the present study. However, it is our intention to provide with these numbers to illustrate the very large number of individuals with S and Z deficiency alleles in these 10 countries, and the need for follow-up epidemiological studies to extend these original observations.

The calculated mean prevalence of AAT deficiency phenotypes carriers [namely MS, SS, MZ, SZ, and ZZ] in FMS patients from these ten selected countries was of 8.9 percent. It should be remarked that the largest percentage [5.2 percent] corresponds to MS phenotypes. Moreover, it would exist in about three percent of SS and MZ moderately deficiency phenotypes and about 0.1 percent of severe deficiency SZ and ZZ phenotypes [Table 4].

Both MS and SS AAT phenotypes are not currently confirmed to be associated with an increased risk for the development of pulmonary and hepatic diseases. The penetrance [number of subjects developing a clinical disease] of both MZ and SZ AAT phenotypes is also clearly lower compared to ZZ (11). So far, the risk for the development of FMS in AAT deficiency patients has not yet been studied. The only existing study in Asturian patients with moderate-severe FMS has shown an intracellular accumulation of AAT, whereas no significant differences were found among the four subgroups [namely MM, MS, MZ, and SZ] regarding the numbers of tender points [mean: 17 ± 1.5], Fibromyalgia Impact Questionnaire

TABLE 3. Estimates of the Prevalences and Numbers of Fibromyalgia Syndrome Patients and Their Alpha-1 Antitrypsin Phenotypic Distribution in Ten Countries

Country total population	PI*S & PI*Z mean gene frequency [$\times 1000$]	FMS mean prevalence [$\times 100$] reference	Calculated number of FMS patients	Estimated prevalence $1/\times$ and total number of subjects in each alpha-1-antitrypsin normal [MM] and five deficiency phenotypic classes [MS, SS, MZ, SZ, and ZZ] with 95% confidence intervals*						Total prevalence and number of deficiency phenotypes
				MM	MS	SS	MZ	SZ	ZZ	
Canada 33,098,932	S: 39 Z: 14	1.1%	251,221	225,813 [223,785-227,704]	1/14 18,601 [16,884-20,475]	1/656 383 [318-460]	1/41 6,131 [5,178-7,249]	1/995 252 [195-326]	1/6,037 42 [30-58]	1/10 25,408 [25,113-25,707]
United States 298,444,215	S: 31 Z: 14	2.0%	5,354,089	4,817,300 [4,785,928-4,847,091]	1/17 323,885 [322,828-324,884]	1/983 5,444 [4,683-6,326]	1/37 143,462 [142,994-143,905]	1/1,110 4,823 [3,988-5,829]	1/5,013 1,068 [849-1,343]	1/11 478,681 [477,388-479,977]
Denmark 5,450,661	S: 28 Z: 27	0.66%	29,247	26,123 [25,943-26,293]	1/19 1,541 [1,415-1,679]	1/1,286 23 [19-27]	1/20 1,495 [1,370-1,630]	1/663 44 [37-52]	1/1,368 21 [18-25]	1/9 3,124 [3,022-3,230]
Finland 5,231,372	S: 7 Z: 7	0.75%	26,170	25,444 [25,231-25,611]	1/69 379 [261-546]	1/18,566 1 [1-3]	1/76 342 [231-502]	1/10,278 3 [1-5]	1/22,758 1 [1-2]	1/36 726 [675-780]
Germany 82,422,299	S: 21 Z: 10	1.13%	779,116	750,593 [745,453-755,268]	1/25 32,500 [28,565-36,493]	1/2,271 352 [274-452]	1/52 15,263 [12,633-18,414]	1/2,418 330 [242-450]	1/10,229 78 [54-112]	1/16 48,523 [48,106-48,944]
Italy 58,133,509	S: 23 Z: 16	2.2%	1,103,723	1,018,847 [1,013,497-1,023,901]	1/23 48,347 [44,379-52,650]	1/1,924 574 [486-677]	1/32 34,831 [31,487-38,513]	1/1,336 826 [689-990]	1/3,708 298 [245-362]	1/13 84,876 [84,328-85,427]
The Netherlands 16,491,461	S: 22 Z: 11	1.15%	146,222	136,583 [135,097-137,883]	1/23 6,351 [5,268-7,641]	1/1,980 74 [51-106]	1/47 3,123 [2,388-4,069]	1/2,014 73 [47-113]	1/8,192 18 [11-30]	1/15 9,639 [9,454-9,827]
Spain 40,397,842	S: 104 Z: 17	2.4%	501,379	387,019 [378,667-394,931]	1/5 91,733 [83,480-100,635]	1/92 5,436 [4,601-6,411]	1/33 15,235 [12,127-19,084]	1/278 1,806 [1,337-2,431]	1/3,344 150 [97-231]	1/4.4 114,359 [113,778-114,943]
Sweden 9,016,596	S: 24 Z: 23	1.3%	97,641	88,576 [86,697-90,159]	1/21 4,554 [3,407-6,048]	1/1,668 59 [33-101]	1/23 4,291 [3,182-5,748]	1/885 110 [63-193]	1/1,879 52 [29-92]	1/11 9,065 [8,889-9,245]
Pakistan 165,803,560	S: 11 Z: 9	2.1%	2,123,944	2,037,979 [1,967,998-2,078,366]	1/46 46,406 [18,570-106,603]	1/8,040 264 [44-1,367]	1/55 38,371 [14,011-96,015]	1/4,824 440 [66-2,462]	1/11,578 183 [25-1,109]	1/25 85,965 [85,403-86,530]

FMS = fibromyalgia syndrome

*95% confidence intervals using Hardy-Weinberg statistics

scores [mean: 67.4 ± 6.1], and Health Assessment Questionnaire scores [mean 1.8 ± 0.6]. However, these subgroups differed significantly in their ages [$P = 0.001$]: MZ subjects [38.5 years] differed significantly from the MM group [51.4 years] and MS group [51.4 years], but not from the SZ group [45.8 years], possibly because of their small sample size.

Recently, evidence from several clinical, epidemiological, and muscle biopsy studies have been found in favor of a relationship between AAT and FMS (16-18). Taken together, these findings would suggest that AAT deficiency

might play an important role in at least a subset of FMS patients, most likely because of the loss of AAT anti-inflammatory efficacy. Other researchers exploring different physiopathological aspects of FMS evidenced a possible relationship between FMS and the increased levels of some inflammatory cytokines [i.e., IL-1, IL-6, IL-8, TNF α] (92-98). Moreover, other studies found a possible relationship between FMS and oxidative stress [in its turn related to microcirculation abnormalities and muscle hypoxia/ischemia] (99-102). It is also worth noting the finding of positive signals of

TABLE 4. Estimated Percentages of Fibromyalgia Syndrome Patients for Each Alpha-1 Antitrypsin Deficiency Phenotype in Ten Countries Worldwide

Country	Calculated number of FMS patients	MS [%]	SS [%]	MZ [%]	SZ [%]	ZZ [%]	Total deficiency phenotypes [%]
Canada	251,221	7.4	0.15	2.4	0.10	0.02	10.1
United States	5,354,089	6.0	0.10	2.7	0.09	0.01	8.9
Denmark	29,247	5.3	0.08	5.1	0.15	0.07	10.7
Finland	26,170	1.4	0.01	1.3	0.01	0.00	2.8
Germany	779,116	4.2	0.04	1.9	0.04	0.10	6.3
Italy	1,103,723	4.4	0.05	3.1	0.07	0.03	7.7
The Netherlands	146,222	4.3	0.05	2.1	0.05	0.01	6.6
Spain	501,379	18.3	1.08	3.0	0.36	0.02	23
Sweden	97,641	4.7	0.06	4.4	0.11	0.05	9.3
Pakistan	2,123,944	2.2	0.01	1.8	0.02	0.01	4.0
Total [mean percent]	10,412,752	[5.2]	[0.16]	[2.7]	[0.10]	[0.03]	[8.9 %]

FMS = fibromyalgia syndrome

IL-1 β , IL-6, and TNF α in fibroblast and mononuclear cells in FMS samples of skin tissues, as well as a neuronal marker [protein gene product 9.5] showing the expression of these cytokines at the site nerves and thus suggesting an inflammatory foci for pain induction (103). In another study (104), advanced glycation end product N-carboxymethyl-lysine, closely related to oxidative stress and tissue damage, was found to be present in the muscle of FMS patients and absent in the muscle biopsies from controls. The N-carboxymethyl-lysine staining was stronger in the interstitial tissue [between muscle fibers] and in the endothelium of small vessels of FMS patients. Moreover, nuclear factor NF-kB and CD68-positive monocytes/macrophages were only demonstrable in the interstitial tissue of FMS patients (104).

The pathogenesis of FMS syndrome still remains unclear, although genetic, environmental, and possibly hormonal factors are suggested to be implicated in its clinical expression. The AAT is a broad-spectrum anti-inflammatory protein with a highly relevant capacity to inhibit most of the inflammatory mediators abnormally increased in FMS syndrome. Previously, it was assumed that the anti-inflammatory effects of AAT were mediated by its anti-protease activity, although recent data suggest that other mechanisms might be involved (105-107). Our own *in vitro* studies, using monocytes stimulated with lipopolysaccharide have shown an inhibition of TNF α production

not only by the native inhibitory form of AAT, but also by AAT chemically modified, non-inhibitory forms, such as polymerized. Furthermore, we have shown that both the native and modified forms of AAT enhance lipopolysaccharide stimulated IL-10 generation (4). Data generated by IL-10 are important, since they infer a specific mechanism for the effects of AAT rather than a general depressive effect of AAT on cell function. Two key anti-inflammatory activities of AAT *in vitro*, namely the inhibition of endotoxin-stimulated TNF α and the enhancement of IL-10 in human monocytes, were found to be mediated by the elevation of the intracellular cyclic adenosine monophosphate and the activation of cyclic adenosine monophosphate dependent protein kinase A. Recently, AAT has also been localized to the surface of vascular endothelium, raising speculation that AAT may modulate endothelial cell function as well as suppress leukocyte serine protease activity (108). We used Affymetrix microarray technology to investigate the effects of AAT on TNF- α stimulated human primary lung microvascular endothelial cell gene expression. In the presence of AAT, the number of genes induced by TNF- α was significantly reduced. In particular, AAT blocked the transcription of TNF- α own gene.

Thus, all these findings lead to the hypothesis that at least a subset of FMS lacking functionally active AAT may suffer from inflammatory processes, mediated by proteases,

cytokines, oxidants, and other inflammation mediators normally regulated by AAT. Therefore, the inherited deficiency of AAT may constitute a genetic modifier of the expression of FMS syndrome.

In conclusion, the suspected role of AAT deficiency in the development of FMS syndrome and the possible efficacy of AAT replacement therapy in severe AAT deficiency patients should warrant the performance of further studies on this area. In this same sense, AAT phenotype characterization in FMS patients should be recommended for a more accurate diagnosis and the management of FMS patients.

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