

Original Article

Genetic epidemiology of alpha-1 antitrypsin deficiency in southern Europe: France, Italy, Portugal and Spain

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Alpha-1-antitrypsin deficiency (AAT deficiency) is one of the most common serious hereditary disorders in the world because it affects all major racial subgroups worldwide and there are at least 120.5 million carriers and deficient subjects worldwide. This genetic disease is related to a high risk for development of jaundice in infants, liver disease in children and adults, and pulmonary emphysema in adults. Moreover, AAT-deficiency carrier phenotypes (PiMS and PiMZ) and deficiency-allele phenotypes (PiSS, PiSZ, and PiZZ) are suspected to make subjects susceptible to a variety of other adverse health effects. As there is a limited database on the number of individuals affected by this disease worldwide, the authors of the present report collected data on control cohorts in genetic epidemiological studies published in the peer-reviewed literature worldwide. The data collected were used to estimate the numbers of carriers and deficiency-allele combinations for the two most common defective alleles, namely PiS and PiZ, in over 58 countries worldwide. The present report focuses on the distribution of the PiS and PiZ deficiency alleles in France, Italy, Portugal, and Spain. The total number of individuals at risk for adverse health effects were as follows: 9, 101, 739 in France; 4, 289, 566 in Italy; 2, 659, 241 in Portugal; and 8, 903, 773 in Spain. The geographical distribution of individual control cohorts and estimates of the numbers of carriers and deficiency-allele phenotypes in each of these four southern European countries are shown in individual tables and maps. This report will be followed by other reports on the remaining countries in Europe, as well as worldwide.

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Alpha-1-antitrypsin deficiency (AAT deficiency) is one of the most common serious hereditary disorders in the world because it affects all major racial subgroups worldwide and there are an estimated 120.5 million carriers and deficient subjects in the 58 countries surveyed worldwide (1).

AAT is a 52,000 molecular-weight alpha-1-glycoprotein, composed of 394 amino acid residues and three asparagine-linked complex carbohydrate side-chains (2). It is produced mainly by hepatocytes and secreted into the blood, where it acts as a circulating serine protease inhibitor whose principal substrate is neutrophil elastase (NE) (3). The AAT gene locus is located on the long arm of chromosome 14, has been mapped to chromosome

14q31-32.3 (4), and is organized into three non-coding (Ia, Ib, and Ic) and four coding (II, III, IV, and V) exons (5). The normal gene is designated PiM, and about 100 normal and defective genetic variants are recognizable by isoelectric focusing (IEF) (6). The two most frequent deficient alleles are PiS (which expresses approximately 50–60% of AAT) and PiZ (which expresses approximately 10–20% of AAT) (4, 7–10).

AAT deficiency is a heritable, autosomal-recessive metabolic disease that results in the synthesis and secretion of defective AAT. Some of the abnormal AAT is retained as pathological polymers within the endoplasmic reticulum of the hepatocytes, resulting in a low plasma

concentration. This deficit is usually insufficient to ensure lifetime protection of the lung from the proteolytic damage of NE, resulting in the early onset of panlobular pulmonary emphysema in adults, especially in habitual tobacco smokers (11–13). In addition, this deficit also can result in the development of neonatal cholestasis, which may progress to infant and juvenile cirrhosis. It also can result in slowly progressive liver disease in up to 50% of the adults so affected, owing to a pathological aggregation of abnormal AAT polymers into hepatocytes (14, 15). On the other hand, AAT deficiency is also suspected to promote asthma (16), bronchiectasis (17), systemic vasculitis (18), relapsing panniculitis (19, 20), rheumatoid arthritis (21), intracranial arterial dissections (22), multiple sclerosis (23), and other diseases. Although some published reviews indicate that both carriers and individuals with deficiency-allele combinations for the PiS- and PiZ-defective alleles (namely PiSS, PiMS, PiMZ, PiSZ, and PiZZ phenotypes) are at risk for several adverse health effects (24–40), clear scientific evidence of the relationship among AAT deficiency and some of these diseases remains to be established.

Knowledge of the prevalence of AAT deficiency in every community is essential for enhancing awareness of this disorder among health-care givers and the general public (6, 41), for planning health policy and financial medical resources and to their utilization by the scientific community, governments, and the pharmaceutical industry.

The present study utilizes data from genetic epidemiological studies performed by others to determine the phenotypes of carriers and deficiency-allele combinations for the two most common defective alleles causing AAT deficiency, namely PiS and PiZ, in the control cohorts of individual case studies from France, Italy, Portugal, and Spain. The data from these individual cohorts for a given country are combined to acquire mean frequencies for the PiM, PiS, and PiZ alleles. These gene frequencies are then utilized to calculate the total numbers of individuals in each of the five major defective phenotypic classes of interest (namely, PiMS, PiMZ, PiSS, PiSZ, PiZZ) in the total population of each of these countries.

The issues that were investigated in the present report are: 'What is the ethnic distribution of AAT deficiency among the European countries, and specifically in France, Italy, Portugal, and Spain?' and 'How many individuals are there in these countries, either carriers or homozygous, for the most common defective alleles for this disease?'

Materials and methods

Sources of the control cohort data used in the present study

The authors of the present manuscript have previously worked independently on the genetic epidemiology of AAT deficiency, and their research has been published in different peer-reviewed journals (1, 42–45). The authors' individual databases were combined to generate a common database used in the present analysis. These articles were obtained through a variety of sources, including searches of the peer-reviewed literature on PubMed and Web of Science in the Library of the National Institutes of Health, and search of reports on genetic epidemiological surveys for AAT deficiency published from 1965 up to August 2002.

Additional relevant studies were obtained by searching bibliographies of the first articles retrieved. Only the data of the control-group cohort phenotypes in each article were used in the present study. Surveys in which phenotypes were identified by selecting sera with AAT serum levels below normal values, and those carried out on hospital-based populations or in patients with AAT deficiency-related diseases (i.e. lung and liver diseases), were omitted, as they could give an excessive number of deficient alleles. The current database on 58 countries worldwide has been combined into 11 geographical regions worldwide. These 11 geographical regions were designated, in part, as an attempt to collate the data on populations with similar anthropological origins (1).

Databases usually consisted of the numbers of control subjects with each of the following phenotypes: PiMM, PiMS, PiMZ, PiSS, PiSZ, PiZZ, and Others. The phenotypes of different AAT alleles were identified by determining their isoelectric point on a thin-layer polyacrylamide gel in a pH gradient of 4–5 (46, 47). Major variation in the migration of various AAT alleles is the result of amino acid substitutions that alter the net charge of the protein and thus the isoelectric point of the protein (48). This technique provides reliable detection of individuals carrying either the PiS or PiZ variant alleles. It is important to note that the *genotype* of individual AAT alleles can only be determined by DNA sequencing (5). There is no evidence that the phenotypic identification of PiS- and PiZ-deficiency alleles by determining their isoelectric points using the IEF technique (46) is complicated by phenocopies (i.e. mutations in other codons that would give a polypeptide chain with isoelectric points identical to those of the PiS and PiZ variants) (46, 48). Therefore, present evidence supports the widespread use of IEF for the rapid, inexpensive,

and critical identification of the PiS and PiZ variants in genetic epidemiological studies of racial and ethnic subgroups worldwide.

These control cohorts (i.e. blood donors, newborns, hospital patients, school or college students, etc.), were described in detail by Hutchinson (47); the same criteria for selection of control cohort data such as sample size, composition, method of analysis of blood samples to detect Pi subtypes, etc. were used in the present study. Articles that reported the total number of subjects in the control cohort, as well as the gene frequencies of PiM, PiS, and PiZ, were also used. Gene frequencies were expressed as the total number of PiS and PiZ, whether in homo- or heterozygotes, per 1000 genes of all Pi types. Using the original data given by the authors on the phenotypes found in each control cohort, 95% confidence intervals (95% CI) were calculated for gene frequencies, gene prevalence, and the numbers of carriers and deficiency-allele combinations.

Estimating gene frequencies of PiM, PiS, and PiZ

The gene frequency ‘y’ was obtained by summing the total number of PiS and PiZ alleles, and expressing this number as a fraction of the total number of Pi alleles in the population (the total number of alleles is twice the number of the subjects). PiS and PiZ 95% CIs of outcomes for each selected survey, for ‘p’ at a significance level of $\alpha < 0.05$, have been calculated using formulas of Documenta Geigy Scientific Tables for a binomial distribution (49). When reported PiS or PiZ frequencies were zero: $X = 0$ (where X represents the number of variant alleles), the lower calculated limit (p_l) was 0 ($p_l = 0$). The upper limit (p_u) was calculated according to the following formula:

$$1 - \text{antilog} \frac{\log \alpha}{N}$$

where N represents the number of alleles studied. When reported frequencies of PiS or PiZ were different from 0 ($X \neq 0$), we applied the following formula:

$$p_l, p_u = \frac{x \mp \frac{1}{2} + \frac{c^2}{2} \mp |c| \sqrt{(x \mp \frac{1}{2}) \left(1 - \frac{x \mp \frac{1}{2}}{N}\right) + \frac{c^2}{4}}}{N + c^2}$$

where c is the typified coordinate at a significance level of α . The prevalence of every phenotype was calculated by applying the Hardy–Weinberg Equilibrium (HWE) principle (50). The Hardy–Weinberg equation is: $p^2 + 2pq + q^2 = 1$ (it equals one because you are summing the frequency of all possible types); source: <http://www.baa.duke.edu>:

16080/BAA93/H-WEQ.HTM. The data on the number of individuals in the total populations in different countries was obtained from two different sources: <http://www.odci.gov/cia/publications/fact-book/index.html> and <http://cnn.countrywatch.com>.

Development of a ‘precision factor score’ of statistical reliability for each control cohort

To assess the statistical reliability of each survey, we developed a new statistical approach to calculate the coefficient of variation (cv) for PiS and PiZ frequencies in each control cohort. This cv gives the precision (or better, the imprecision) of results from each survey. Imprecision measures the dispersion of the data in respect to the mean, and it depends on the total number of studied alleles and on the gene frequencies of PiS and PiZ actually found. The precision is inversely proportional to the values of cv , so the smaller the value of cv the greater the precision. The 95% CI of the gene frequencies for PiS and PiZ for a given control cohort are large when the gene frequencies are small. Therefore, a reliable study depends on the number of the subjects in each control cohort, as well as the gene frequencies of PiS and PiZ (43).

Numerical precision factor scores (PFS) for assessing the statistical quality and precision (or imprecision) of each selected survey were generated as follows: from both S and Z cv s:

$$Zcv = \frac{100 \times (Zul - Zll)}{4 \times Zfr}, \text{ and}$$

$$Scv = \frac{100 \times (Sul - Sll)}{4 \times Sfr}$$

The mean cv value (\bar{cv}) was calculated by using the following formula:

$$\bar{cv} = \frac{Zcv + Scv}{2}$$

and the numerical PFS was calculated by using the following formula:

$$\text{PFS} = 500 \times \frac{1}{\bar{cv}},$$

(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 for obtaining a value scale for the PFS from approximately 0 to 12).

These statistical calculations provide estimates of the mean, median, standard deviation, and the

range (upper and lower values) of the PFS in each of the control cohorts for a given country.

Development of Microsoft Excel templates with imbedded commands for automated database processing

Microsoft Excel spreadsheets were developed to record and process the data from genetic epidemiological studies to determine Pi types in control populations in each country. The data from surveys within a country were tabulated individually to determine the total numbers in each of eight different genotypic classes, namely PiMM, PiMS, PiMZ, PiSS, PiSZ, PiZZ, PiM-, and Others. The data in each report on the total number of subjects and the numbers in each of these eight phenotypic classes were used to calculate gene numbers for PiM, PiS, and PiZ and in the gene frequencies for PiM, PiS, and PiZ for the entire country. Formulas were developed and imbedded into Microsoft Excel spreadsheet templates to process the original control cohort data in individual reports to calculate a derived database consisting of the numbers of genes as well as the mean gene frequencies for PiM, PiS, and PiZ. These formulas calculate: the total number of cohorts, the total number of subjects in all cohorts and the total numbers in each of the above eight phenotypic classes; the total gene numbers for PiM, PiS and PiZ and their frequencies with 95% CI; estimates of the prevalence of heterozygotes (PiMS and PiMZ) and deficiency-allele combinations (PiSS, PiSZ, and PiZZ), using HWE statistics and 95% CI in each country; and scores for assessing the statistical quality of every survey and all surveys from every country.

The Microsoft Excel spreadsheet was also designed to then compare the numbers in the original and derived databases so that error messages would be generated to highlight any discrepancy between the original and derived numbers as well as to guard against accidental erroneous data entry for any given control cohort. We assumed that if these formulas could successfully recreate the number of subjects in each of these eight

phenotypic classes in the derived database then the derived gene frequencies for PiM, PiS, and PiZ could be used with HWE statistics to estimate the total number of carriers (PiMS and PiMZ) and deficiency-allele combinations (PiSS, PiSZ, and PiZZ) for each country.

Spreadsheets were linked to each of the original country data sheets so that these numbers would change automatically with any new cohort entry into the individual country spreadsheet. The numbers are summarized in the spreadsheet for each geographical region to estimate the total numbers of carriers and deficiency-allele combinations for PiS and PiZ.

The primary database on the individual countries in the present report provides the supporting documentation for each of the geographical regions. It is the intention of the authors to update each country database on an Internet website as new papers on such genetic epidemiological surveys are published in countries already included, as well as reports on new countries as they emerge. This new type of follow-up publication provides a mechanism to ensure that all data is current.

Results

Overview

Our database searches on each of the four southern European countries included in the study provided data on 92 cohorts with a total of 41,079 individuals. These 92 cohorts are distributed as follows: 20 from France; 40 from Italy; four from Portugal; and 28 from Spain. Calculated statistical values of the PFS for the control cohort samples from each of the four countries are shown in Table 1. The database for each of the four countries is given in Tables 2–5. Results are represented graphically in Figs 1–4. Summary data of the four countries are shown in Tables 6 and 7.

France

Overview. A total of 20 control cohorts were selected from studies performed in France (51–62). Results are shown in Table 2 and Fig. 1.

Table 1. Calculated precision factor scores (PFS) of the control cohorts in four countries in southern Europe

Country	PFS mean	Cohort number	Mean	SD	Median	Range (lower limit)	Range (upper limit)	Total cohort size
France	7.4	20	619	524	394	100	1653	11,978
Italy	5.0	40	470	176	202	65	7522	17,453
Portugal	7.3	4	372	372	274	39	900	1488
Spain	6.2	28	277	219	170	56	1116	7763
Total	6.0	92	394	787	201	14	7522	42,099

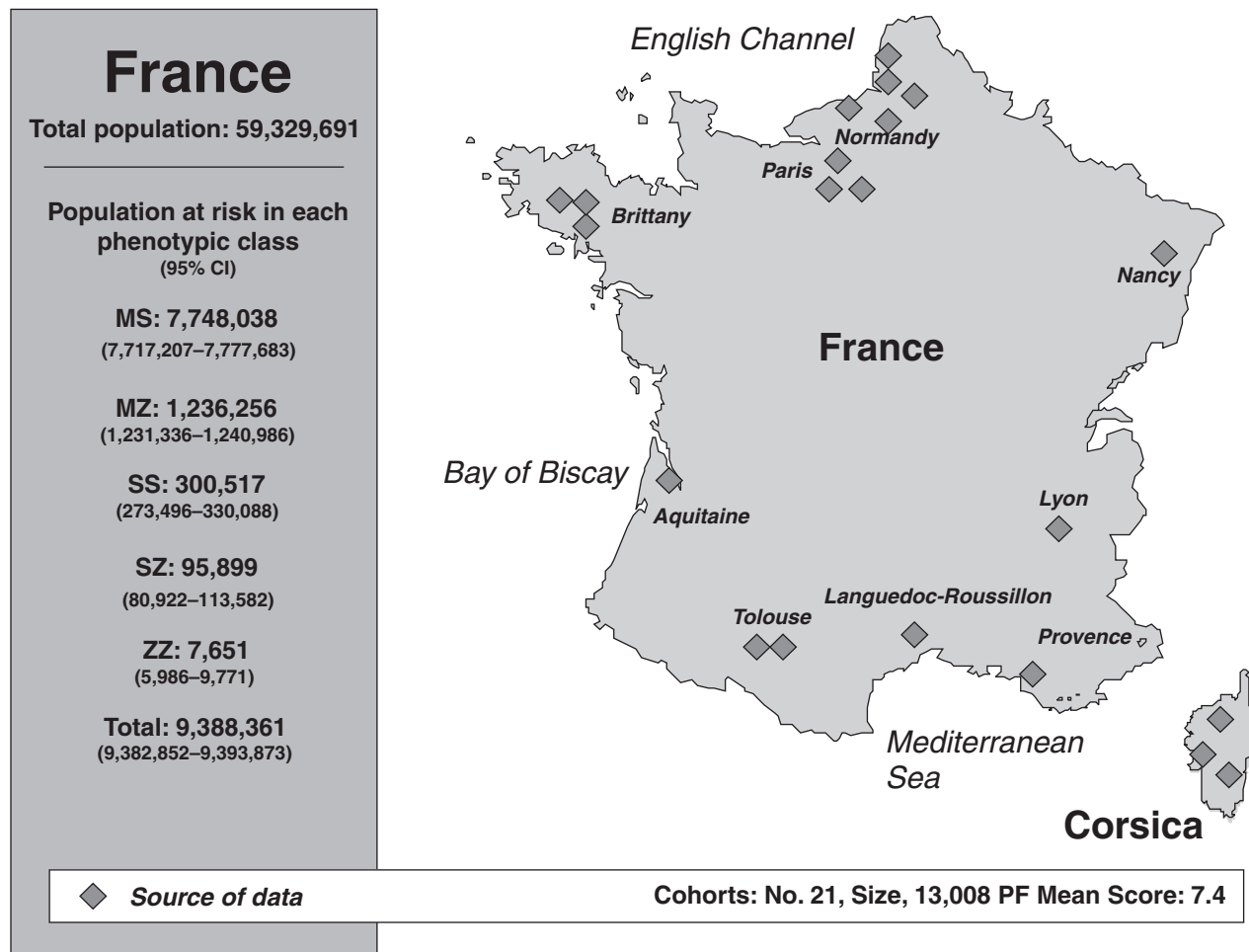


Fig. 1. France: number and geographic location of control cohorts and the number of the 5 phenotypic classes of carriers and deficiency allele combinations with 95% confidence intervals.

Regions that supplied the data for the present research are located in the north, south and west of the country. Three out of the 20 cohorts are from Corsica. Noteworthy is the remarkable lack of studies from the central areas of France.

The number of individuals studied was 11,978 in a total population of 59,329,691 inhabitants. Samples were obtained from blood donors, workers, cohorts of unrelated healthy people, controls, and natives from Brittany, Normandy, and Corsica. None of these studies was made on the general population selected at random.

Calculation of the PFS for each control cohort.

Calculated statistical values of the PFS were: mean 7.4, median 7.0, SD 3.03, and range 3.0–12.6. Sixteen out of 20 studies yielded a high PFS score (approximately 5–12). Ten of these 16 reliable studies were carried out in healthy unrelated persons (HUP), most of whom were workers from Paris (51), Nancy (51), Bordeaux (51), Normandy (56), Languedoc-Roussillon (57), Lyon (58), and Toulouse (51,60). The remaining six

reliable studies were carried out in unrelated healthy people: four in Normandy (51, 54, 55, 61) and two in Brittany (52, 53). The remaining studies, including those carried out in Corsica, showed lower scores, and they should be considered with caution.

Estimation of gene frequencies. The mean frequency for the PiS allele was 69.0 (66–72) per 1000, and the mean gene frequency for PiZ was 10.9 (9.7–12.4) per 1000. The maximal gene frequencies for PiS were observed in southern France, showing a gradual decrease to the eastern and northern regions all over the country. Maximal PiZ values were observed in western regions, with maximal values (about 22–23 per 1000) in Aquitaine, Brittany and Normandy, and showing a gradual decrease to the east.

Estimation of gene prevalence. Estimates of Pi gene frequency using HWE statistics indicate that there is one carrier- or deficiency-allele combination phenotype for every 6.5 individuals in the French

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Table 2. Gene frequencies of PIS and PIZ (per 1000) and prevalence of Pi phenotypes MM, MS, MZ, SS, SZ and ZZ in 20 control cohorts in France in a total population of 59,329,691

Author, year, (reference)	Region, ethnic subgroup	No., type, method, PFS	Gene frequency (per 1000)			Calculated prevalence of Pi Phenotypes 1/x (Hardy-Weinberg Equilibrium statistics)					
			PIS	PIZ	MM	MS	MZ	SS	SZ	ZZ	
Mainland											
Sesboue & Martin, 1991 (51)	Ile-de-France, Paris-Clichy	371, HUP, IEF, 6.7	90 (71-114)	7 (2-17)	1.23	6	82	123	822	22,023	
Sesboue & Martin, 1991 (51)	Ile-de-France, Paris-Creteil	151, HUP, IEF, 5.2	76 (50-114)	13 (4-36)	1.21	7	41	172	496	5700	
Sesboue & Martin, 1991 (51)	Ile-de-France, Pais Villejuif	719, HUP, IEF, 9.0	84 (71-100)	11 (7-18)	1.22	7	50	141	534	8078	
Sesboue & Martin, 1991 (51)	Lorraine, Nancy	1,551, HUP, IEF, 10.8	52 (44-60)	11 (8-15)	1.14	10	49	371	879	8324	
Sesboue et al., 1978 (52)	Brittany, Morbihan	280, HUP, IEF, 7.0	75 (55-101)	23 (13-40)	1.23	7	24	178	287	1856	
Youinou et al., 1984 (53)	Brittany, Breton (Bigoudens)	397, HUP, IEF, 7.2	63 (48-83)	16 (9-29)	1.18	9	33	252	485	3730	
Youinou et al., 1984 (53)	Brittany, Breton (non-Bigoudens)	100, HUP, IEF, 3.9	60 (33-105)	5 (0-32)	1.14	9	107	278	1667	40,000	
Sesboue & Martin, 1991 (51)	Normandy, Rouen	419, HUP, IEF, 6.6	68 (52-88)	7 (3-16)	1.17	8	76	216	1027	19,507	
Morcamp et al., 1980 (55)	Normandy, Rouen	1030, HUP, CAAE, 10.6	63 (53-75)	18 (13-25)	1.18	9	30	251	441	3100	
Vandeville et al., 1972 (56)	Normandy, Seine-Maritime	944, HUP, CAAE, 5.2	19 (14-27)	1 (0-4)	1.04	27	482	2750	24,754	891,136	
Sesboue & Martin, 1991 (51)	Aquitaine, Bordeaux	356, HUP, IEF, 8.5	125 (102-152)	22 (13-37)	1.38	5	26	64	178	1980	
Robinet-Levy & Reunier, 1972 (57)	Languedoc-Roussillon	1520, HUP, CAAE, 10.8	79 (70-89)	6 (3-9)	1.19	7	98	160	1133	31,978	
Sesboue & Martin, 1991 (51)	Provence, Marseille	239, HUP, IEF, 4.5	38 (23-60)	4 (1-17)	1.09	14	125	705	3173	57,121	
Arnaud et al., 1977 (58)	Rhone-Alpes, Lyon	1653, HUP, IEF, 12.6	71 (63-81)	14 (11-19)	1.20	8	38	196	493	4,948	
Constans et al., 1980 (60)	Midi-Pyrenes, Toulouse	163, HUP, CAAE, 5.5	141 (106-185)	0 (0-11)	1.36	4	Infinite	50	Infinite	Infinite	
Sesboue & Martin, 1991 (51)	Midi-Pyrenes, Toulouse	1247, HUP, IEF, 12.2	105 (93-118)	13 (9-18)	1.29	5	44	91	371	6074	
Corsica											
Memmi et al. 1998 (59)	Corsica, Ajaccio	153, HUP, IEF, 3.0	0 (0-12)	0 (0-12)	1.00	Infinite	Infinite	Infinite	Infinite	Infinite	
Moral et al. 1996 (62)	Corsica, Corte	167, HUP, IEF, 3.3	9 (2-28)	0 (0-11)	1.02	56	Infinite	12,395	Infinite	Infinite	
Moral et al. 1997 (87)	Corsica, Bastia	124, HUP, IEF, 3.6	28 (12-60)	0 (0-15)	1.06	18	Infinite	1255	Infinite	Infinite	

No., number of subjects; Type: HUP, healthy unrelated persons (includes blood donors, workers, controls, subjects not clearly specified, etc.); Method: IEF, isoelectrofocusing; CAAE, crossed antigen-antibody electrophoresis; PFS, precision factor score.

Table 3. Gene frequencies PIS and PiZ (per 1000) and the prevalence of Pi Phenotypes MM, MS, MZ, SS, SZ and ZZ in 40 control cohort populations in Italy (Total population of 57 634 327)

Author, year, (reference)	Region, ethnic subgroup	No., type, method, PFS	Gene frequency (per 1000)		Calculated prevalence of Pi Phenotypes 1/x (Hardy-Weinberg Equilibrium statistics)						
			PIS	PiZ	MM	MS	MZ	SS	SZ	ZZ	
Mainland											
Klasen et al., 1982 (70)	Northern Italy	202, HUP, IEF, 4.6	30 (16-53)	10 (3-27)	1.08	18	53	1133	1700	10,201	
Pittschieler & Massi 1988 (63)	South Tyrol Germans	7522, NB, IEF, 19.2	15 (13-17)	20 (17-22)	1.07	36	26	4719	1758	2618	
Pittschieler & Massi 1988 (63)	South Tyrol Italians	1606, NB, IEF, 10.6	32 (26-39)	15 (11-20)	1.10	17	35	992	1054	4478	
Klasen et al., 1978 (94)	Venetia, Verona	202, HUP, IEF, 4.6	30 (16-53)	10 (3-27)	1.08	18	53	1133	1700	10201	
Paoli et al., 1993 (95)	Venetia, Padua	98, HUP, IEF, 3.4	20 (7-55)	5 (0-32)	1.05	25	101	2401	4802	38,416	
Seefried-Lehmann et al., 1992 (68)	Venetia, Rovigo	81, HUP, IEF, 3.3	31 (11-74)	0 (0-23)	1.06	17	Infinite	1050	Infinite	Infinite	
Seefried-Lehmann et al., 1992 (68)	Venetia, Treviso	109, HUP, IEF, 3.7	18 (6-49)	9 (2-36)	1.06	28	56	2970	2970	11,881	
Seefried-Lehmann et al., 1992 (68)	Venetia, Venice	117, HUP, IEF, 3.9	43 (22-80)	4 (0-27)	1.10	12	123	548	2738	54,756	
Seefried-Lehmann et al., 1992 (68)	Liguria, Genoa	208, NB, IEF, 4.8	50 (32-77)	12 (4-29)	1.14	11	44	392	824	6922	
Seefried-Lehmann et al., 1992 (68)	Piamonte, Turin	394, NB, IEF, 5.7	32 (21-47)	9 (4-19)	1.09	16	59	994	1774	12,672	
Massi et al., 1982 (69)	Piamonte, Cuneo	208, NB, IEF, 5.3	55 (36-83)	5 (1-19)	1.13	10	111	327	1881	43,264	
Seefried-Lehmann et al., 1992 (68)	Emilia Romagna, Bologna	166, HUP, IEF, 4.2	24 (11-49)	9 (2-28)	1.07	21	57	1722	2296	12,247	
Seefried-Lehmann et al., 1992 (68)	Emilia Romagna, Ferrara	277, HUP, IEF, 4.1	25 (14-43)	2 (0-12)	1.06	20	285	1566	10,961	306,916	
Seefried-Lehmann et al., 1992 (68)	Emilia Romagna, Forli	232, HUP, IEF, 4.0	28 (16-49)	2 (0-14)	1.06	18	239	1274	8281	215,296	
Seefried-Lehmann et al., 1992 (68)	Emilia Romagna, Modena	65, HUP, IEF, 3.7	31 (10-82)	15 (3-60)	1.10	17	34	1056	1056	4225	
Seefried-Lehmann et al., 1992 (68)	Emilia Romagna, Ravenna	155, HUP, IEF, 3.5	16 (6-39)	3 (0-21)	1.04	32	158	3844	9610	96,100	
Massi et al., 1982 (69)	Emilia Romana, Parma	268, HUP, IEF, 5.1	24 (14-42)	13 (6-28)	1.08	21	40	1700	1579	5863	
Massi et al., 1982 (69)	Emilia-Romana, Bologna	263, HUP, IEF, 4.3	13 (6-28)	8 (2-21)	1.04	38	67	5646	4941	17,292	
Walter et al., 1989 (67)	Tuscany, Lucca	172, HUP, IEF, 3.8	17 (7-39)	6 (1-23)	1.05	29	88	3287	4931	29,584	
Massi et al., 1982 (69)	Tuscany, Livorno	490, HUP, IEF, 5.8	30 (20-43)	7 (3-15)	1.08	18	73	1142	2366	19,600	
Massi et al., 1982 (69)	Tuscany, Grosseto	172, NB, IEF, 3.6	17 (7-39)	3 (0-19)	1.04	29	176	3287	9861	118,336	
Massi et al., 1982 (69)	Tuscany, Arezzo	472, HUP, IEF, 5.7	36 (25-51)	5 (2-13)	1.09	14	98	771	2621	35,645	

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Piantelli et al., 1978 (64)	Latium, Rome	500, NB, IEF, 7.8	67 (53-85)	15 (9-25)	1.19	8	36	223	498	4444
Pascali & Demercurio, 1981 (65)	Latium, Rome	513, HUP, IEF, 6.2	28 (19-41)	10 (5-18)	1.08	18	53	1252	1815	10,527
Massi et al., 1980 (66)	Latium, Rome	967, NB, IEF, 8.6	44 (35-54)	11 (7-17)	1.12	12	49	518	1048	8482
Klasen, 1981 (96)	Campania, Naples	150, HUP, IEF, 4.4	27 (12-54)	13 (4-36)	1.09	20	39	1406	1406	5625
Klasen et al., 1982 (70)	Campania, Naples	260, NB, IEF, 5.0	25 (14-43)	12 (5-26)	1.08	21	45	1600	1733	7511
Seefried-Lehmann et al., 1992 (68)	Campania, Salerno	247, HUP, IEF, 3.4	4 (1-16)	0 (0-7)	1.01	124	Infinite	61 009	Infinite	Infinite
Massi et al., 1982 (69)	Molize, Campobasso	600, NB, IEF, 5.5	27 (19-38)	3 (1-9)	1.06	19	155	1406	5625	90,000
Seefried-Lehmann et al., 1992 (68)	Apulia, Bari	420, HUP, IEF, 3.7	10 (4-19)	1 (0-8)	1.02	53	425	11,025	44,100	705,600
Seefried-Lehmann et al., 1992 (68)	Calabria, Cosenza	96, HUP, IEF, 2.8	0 (0-19)	0 (0-19)	1.00	Infinite	Infinite	Infinite	Infinite	Infinite
Seefried-Lehmann et al., 1992 (68)	Calabria, Reggio Calabria	188, HUP, IEF, 3.5	11 (3-29)	0 (0-10)	1.02	48	Infinite	8836	Infinite	Infinite
Sardinia										
Davrinche et al., 1984 (97)	Sardinia, Cagliari	218, HUP, IEF, 4.9	62 (42-90)	5 (1-18)	1.15	9	117	261	1760	47,524
Massi et al., 1982 (69)	Sardinia, Cagliari	100, NB, IEF, 3.2	15 (4-47)	0 (0-18)	1.03	34	Infinite	4444	Infinite	Infinite
Moral et al., 1994 (98)	Sardinia, Cagliari	148, HUP, IEF, 3.6	20 (8-46)	0 (0-12)	1.04	25	Infinite	2434	Infinite	Infinite
Walter et al., 1989 (67)	Sardinia, Cagliari	272, HUP, IEF, 5.2	44 (29-66)	7 (2-20)	1.11	12	72	514	1541	18,496
Walter et al., 1989 (67)	Sardinia, Sassari	239, HUP, IEF, 4.9	36 (22-57)	8 (3-23)	1.09	15	62	791	1680	14,280
Walter et al., 1989 (67)	Sardinia, Nuoro	79, HUP, IEF, 3.4	38 (16-85)	0 (0-23)	1.08	14	Infinite	693	Infinite	Infinite
Walter et al., 1989 (67)	Sardinia, Oristano	169, HUP, IEF, 4.4	41 (24-70)	6 (1-24)	1.10	13	89	583	2040	28,561
Sicily										
Massi et al., 1982 (69)	Sicily, Palermo	175, NB, IEF, 4.1	17 (7-39)	9 (2-27)	1.05	30	60	3403	3403	13,611

No., number of subjects; Type: HUP, healthy unrelated persons (includes blood donors, workers, controls, subjects not clearly specified, etc.); NB, newborns; Method: IEF, isoelectrofocusing; PFS, precision factor score.

Table 4. Gene frequencies of PiS and PiZ (per 1000) and prevalence of Pi phenotypes MM, MS, MZ, SS, SZ and ZZ in four control cohorts in Portugal in a total population of 10,048,232

Author, year, (reference)	Region, ethnic subgroup	No., type, method, PFS	Gene frequency (per 1000)			Calculated prevalence of Pi Phenotypes 1/x (Hardy-Weinberg Equilibrium statistics)				
			PiS	PiZ	MM	MS	MZ	SS	SZ	ZZ
Geada et al., 1976 (72)	Lisbon, Students	219, HUP, CAAE, 6.0	55 (36-82)	23 (12-43)	1.18	10	24	333	400	1918
Fagerhol & Terifjord, 1968 (75)	Lisbon, Seamen	39 HUP, CAAE, 3.7	141 (76-243)	0 (0-46)	1.36	4	Infinite	50	Infinite	Infinite
Martin et al., 1976 (73)	Lisbon	330, HUP, CAAE, 7.8	115 (92-143)	18 (10-32)	1.33	5	32	75	239	3025
Santos-Rosa & Robalo-Cordeiro, 1986 (74)	All Regions	900, HUP, IEF, 11.6	152 (136-169)	10 (6-16)	1.42	4	60	43	330	10,000

No., number of subjects, Type: HUP, healthy unrelated persons (includes blood donors, workers, controls, subjects not clearly specified, etc.); Method: IEF, isoelectrofocusing; CAAE, crossed antigen-antibody electrophoresis; PFS, precision factor score.

Table 5. Gene frequencies of PIS and PIZ (per 1000) and prevalence of Pi phenotypes MM, MS, MZ, SS, SZ and ZZ in 28 control cohort populations in Spain (total population 39,996,671)

Author, year, (reference)	Region, ethnic subgroup	No., type, method, PFS	Gene frequency (per 1000)		Calculated prevalence of Pi Phenotypes 1/x (Hardy-Weinberg Equilibrium statistics)						
			PIS	PIZ	MM	MS	MZ	SS	SZ	ZZ	
Mainland											
Goedde et al., 1973 (76)	Basque Country, Pamplona & Bermeo	146, HUP, XAA, 5.3	116 (83-160)	7 (1-27)	1.30	5	83	74	627	21,316	
Estefania et al., 1987 (89)	Basque Country, Biscay, Bilbao	166, HUP, IEF, 6.0	117 (85-158)	15 (6-37)	1.33	5	38	73	285	4444	
Estefania et al., 1987 (89)	Basque Country, Biscay, Bilbao	56, HUP, IEF, 3.8	107 (59-183)	0 (0-32)	1.25	5	Infinite	87	Infinite	Infinite	
Estefania et al., 1987 (89)	Basque Country, Biscay, Bilbao	390, HUP, IEF, 7.1	108 (88-132)	6 (2-15)	1.27	5	94	86	772	27,778	
Estefania et al., 1987 (89)	Basque Country, Alva, San Sebastian	155, HUP, IEF, 5.2	126 (92-169)	0 (0-12)	1.31	5	Infinite	63	Infinite	Infinite	
Estefania et al., 1987 (89)	Basque Country, Guipuzcoa, Vitoria	99, HUP, IEF, 4.7	132 (90-189)	5 (0-32)	1.34	4	116	57	758	40,000	
Manzano et al., 1993 (81)	Alava, Basque Country	323, HUP, IEF, 5.8	101 (79-127)	2 (0-10)	1.24	6	360	99	3210	417,316	
Garcia-Orad et al., 1990 (84)	Basque Country, Vizcaya and Guipuzcoa	437, HUP, IEF 7.5	100 (81-122)	8 (4-17)	1.26	6	70	101	627	15,589	
Blanco et al., 1999 (42)	Asturias, High and Middle Naron basins	1116, GP, IEF, 12.4	100 (88-113)	20 (15-27)	1.29	6	29	100	254	2573	
Goedde et al., 1973 (76)	Galicia, Lugo	129, HUP, XAA, 5.2	147 (108-198)	4 (0-25)	1.39	4	152	46	876	66,564	
Caeiro, 1983 (77)	Galicia, Lugo	496, HUP, IEF, 8.6	139 (118-163)	8 (4-16)	1.37	4	73	52	446	15,376	
Carracedo & Concheiro, 1983 (82)	Galicia, Regional districts	480, HUP, IEF, 5.6	149 (127-173)	9 (5-18)	1.41	4	63	45	358	11,378	
Fagerhol & Teniford, 1968 (75)	Spanish seaman, Galicia	378, HUP, XAA, 7.7	112 (91-138)	12 (6-23)	1.30	5	48	79	374	7056	
Vidal1 et al., 1996 (79)	Catalonia, Barcelona	440, HUP, IEF, 8.3	105 (86-127)	16 (9-27)	1.29	5	36	91	301	3951	
Moreno & Moral, 1988 (92)	Catalonia, Girona	291, HUP, IEF, 5.7	74 (55-99)	5 (1-16)	1.18	7	105	183	1313	37,636	

Table 5. Continued

Author, year, (reference)	Region, ethnic subgroup	No., type, method, PFS	Gene frequency (per 1000)		Calculated prevalence of Pi Phenotypes 1/x (Hardy-Weinberg Equilibrium statistics)						
			PIS	P1Z	MM	MS	MZ	SS	SZ	ZZ	
Puertas & Arranz Pena, 1989 (88)	Castilla-Leon, Valladolid	457, NB, IEF, 9.4	135 (113-159)	21 (13-33)	1.40	4	28	55	179	2314	
Klassen et al., 1980 (80)	Madrid, La Paz	103, Hosp, IEF, 4.2	83 (50-131)	0 (0-18)	1.19	7	Infinite	147	Infinite	Infinite	
Goedde et al., 1973 (76)	Central Meseta, Madrid	170, HUP, XAA, 5.0	97 (69-135)	0 (0-11)	1.23	6	Infinite	106	Infinite	Infinite	
Moral1 et al., 1994 (98)	Central Spain, South slope, Sierra de Gredos	97, HUP, IEF, 4.3	88 (53-139)	5 (0-33)	1.22	6	107	130	1107	37,636	
Moral et al., 1994 (78, 98)	Central Spain, North slope, Sierra de Gredos	106, HUP, IEF, 4.5	108 (71-160)	0 (0-17)	1.26	5	Infinite	85	Infinite	Infinite	
Moreno & Horro, 1991 (85)	Central Pyrenes, Sobrarbe and Ribagorza	146, HUP, IEF 5.3	147 (110-194)	0 (0-13)	1.38	4	Infinite	46	Infinite	Infinite	
Moral et al., 1986 (90)	Catalonia, Pyrenees	170, HUP, IEF, 2.7	141 (107-184)	0 (0-11)	1.36	4	Infinite	50	Infinite	Infinite	
Perez-Gutierrez et al., 1989 (86)	Oriental Andalusia	394, HUP, IEF, 5.7	76 (59-97)	1 (0-8)	1.17	7	427	172	5175	620,944	
Goedde et al., 1973 (76)	Andalusia, Seville	131, HUP, XAA, 5.2	99 (67-144)	11 (3-36)	1.26	6	49	102	440	7,627	
Prados et al., 1988 (91)	SW Spain, SW Andalucla	200, HUP, IEF, 5.2	90 (65-123)	0 (0-9)	1.21	6	Infinite	123	Infinite	Infinite	
Balearic Islands											
Moral et al., 1986 (90)	Balearic Islands, Minorca	445, HUP, IEF, 7.3	83 (66-104)	9 (4-18)	1.21	7	61	145	669	12,346	
Picornell et al., 1992 (83)	Balearic Islands, Majorca, Jewish Cheutas	134, HUP, IEF, 4.1	52 (30-88)	4 (0-24)	1.12	10	142	366	2565	71,824	
Canary Islands											
Moral et al., 1997 (87)	Canary Islands, Tenerife	108, HUP, IEF, 5.2	144 (101-199)	9 (2-37)	1.39	4	64	49	376	11,664	

No., number of subjects; Type: HUP, healthy unrelated persons (includes blood donors, workers, controls, subjects not clearly specified, etc.); GP, general population; Hosp., Hospital patients; NB, newborns; Method: IEF, isoelectrofocusing; CAAE, crossed antigen-antibody electrophoresis; PFS, precision factor score.

Genetic epidemiology of AAT deficiency in southern Europe

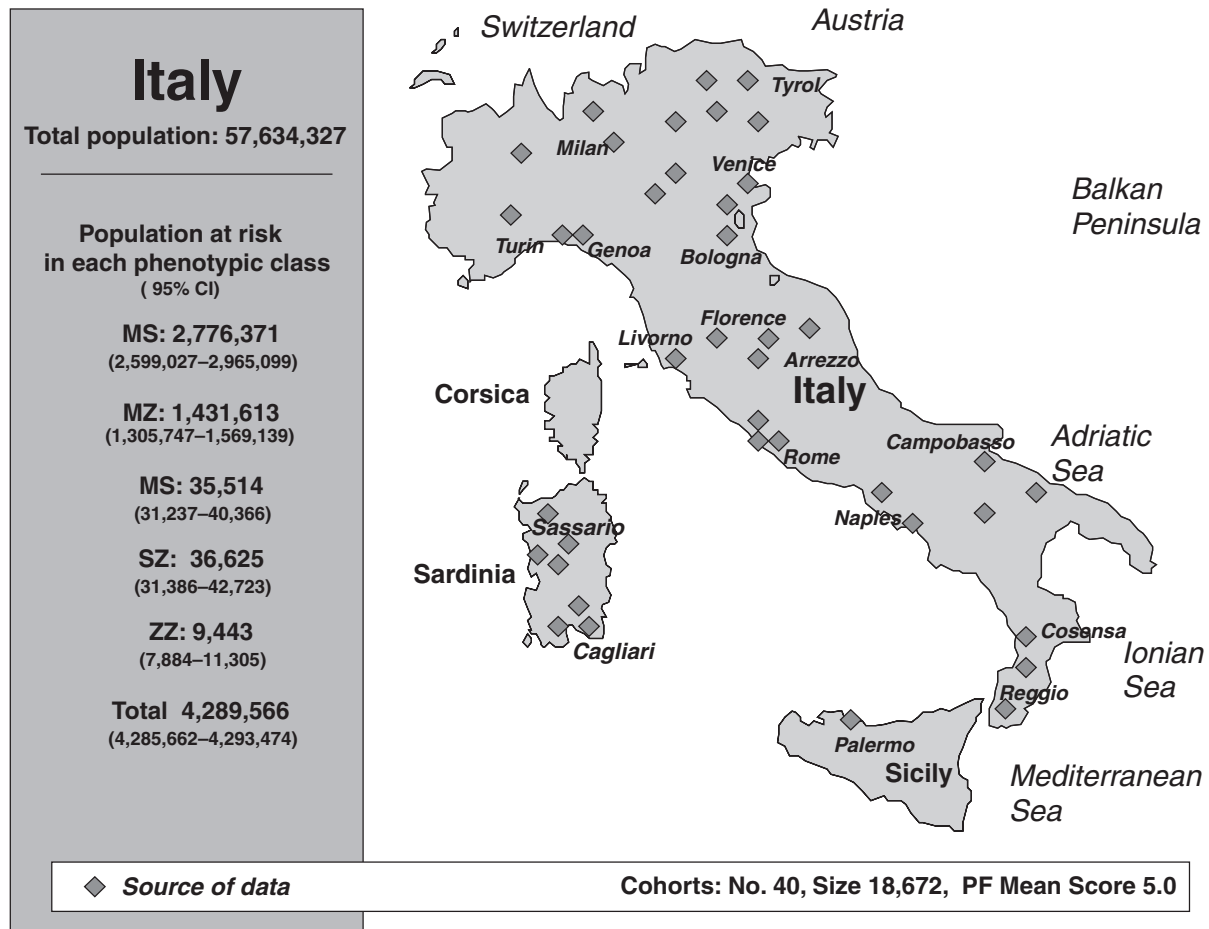


Fig. 2. Italy: number and geographic location of control cohorts and the number of the 5 phenotypic classes of carriers and deficiency allele combinations with 95% confidence intervals.

population, with a rate of one in eight of phenotype PiMS, one in 50 of phenotype PiMZ, one in 210 of phenotype PiSS, one in 663 of phenotype PiSZ, and one in 8360 of phenotype PiZZ.

Estimation of the numbers of carriers (PiMS and PiMZ) and deficiency-allele combinations (PiSS, PiSZ, and PiZZ). Estimates of the numbers of carriers and deficiency-allele combinations using HWE statistics indicate a total of 9,101,739 (95% CI: 9,096,299–9,107,181) individuals at risk, with 7,528,952 (95% CI: 7,156,388–7,918,242) having phenotype PiMS; 1,194,059 (95% CI: 1,052,395–1,354,038) having phenotype PiMZ; 282,139 (95% CI: 256,870–309,785) having phenotype PiSS; 89,492 (95% CI: 75,549–105,948) having phenotype PiSZ; and 7097 (95% CI: 5555–9059) having phenotype PiZZ.

The estimates of the numbers of carriers and deficiency-allele combinations using HWE statistics are influenced by the very different gene frequencies for PiS and PiZ on the island

of Corsica, where the population size is 260,700 (<http://www.francekeys.com>).

If this number is subtracted from the total population of France, the numbers of carriers and deficiency-allele combinations are higher, with a total of 9,348,527 (95% CI: 9,343,030–9,354,027) individuals at risk: 7,715,082 (95% CI: 7,331,594–8,115,793) of phenotype PiMS; 1,231,045 (95% CI: 1,084,862–1,396,138) of phenotype PiMZ; 299,286 (95% CI: 272,432–328,667) of phenotype PiSS; 95,509 (95% CI: 80,623–113,077) of phenotype PiSZ; and 7620 (95% CI: 5965–9726) of phenotype PiZZ.

A weighted-mean approach can be taken by using the data from France as well as Corsica by adjusting the data for the percentages of these two populations in the total population of France. This exercise provides the numbers of carriers and deficiency-allele combinations as follows, with a total of 9,388,468 (95% CI: 9,382,852–9,393,873) individuals at risk: 7,748,038 (95% CI: 7,717,207–7,777,683) of phenotype PiMS; 1,236,256 (95% CI: 1,231,336–1,240,986) of

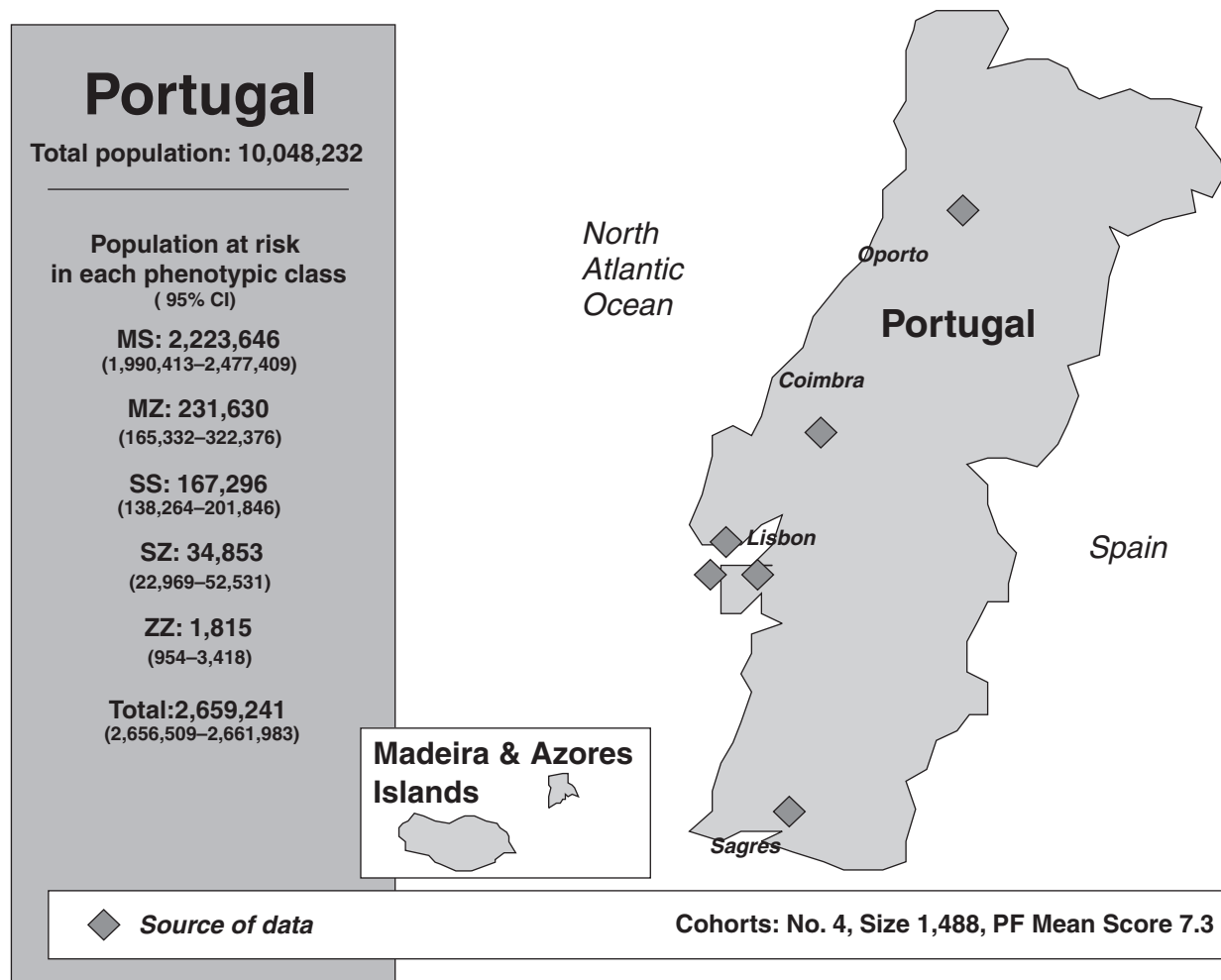


Fig. 3. Portugal: number and geographic location of control cohorts and the number of the 5 phenotypic classes of carriers and deficiency allele combinations with 95% confidence intervals.

phenotype PiMZ; 300,517 (95% CI: 273,496–330,088) of phenotype PiSS; 95,899 (95% CI: 80,922–113,582) of phenotype PiSZ; and 7651 (95% CI: 5986–9771) of phenotype PiZZ.

This type of analysis demonstrates that when it is possible to obtain estimates of the size of a subgroup population that the weighted-mean approach provides the most accurate estimates of the numbers of carriers and deficiency-allele combinations.

Italy

Overview. A total of 40 control cohorts were selected from studies performed in Italy (58–70). Results are shown in Table 3 and Fig. 2. Regions that supplied the data for the present research are located in the north, center and south of the Italian peninsula. Moreover, seven populations were obtained from the Island of Sardinia and one from the Island of Sicily.

The number of individuals studied was 18,061 for a total population of 57,634,327 inhabitants. Most samples were controls (HUP), natives from Sardinia, and newborn infants. None of these studies was made on the general population selected at random.

Calculation of the PFS for each control cohort. Calculated statistical values of the PFS were: mean 5.0, median 4.4, SD 2.8, and range 2.8–19. Italian surveys showed very erratic scores. So, while two surveys carried out in newborns from the South Tyrol (63) gave the highest score found in all four countries from southern Europe, scores for the majority of the remaining Italian surveys (including most of those from Sicily and Sardinia) were generally low, with the exception of 10 surveys performed in Rome (64–66), Sardinia (67), Genoa (68), Livorno (69), Parma (66), Arrezzo (66), and southern Italy (70, 71).

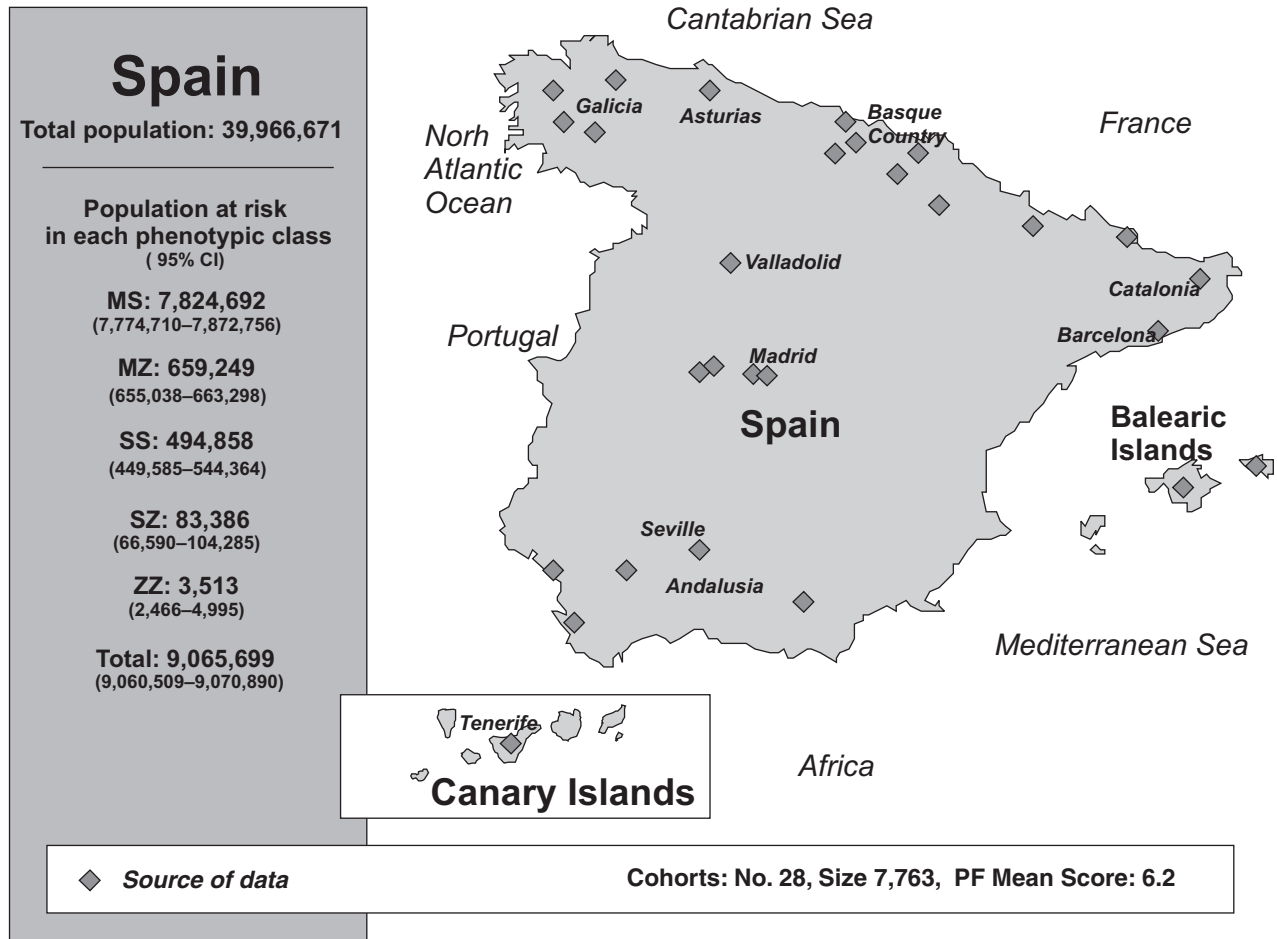


Fig. 4. Spain: number and geographic location of control cohorts and the number of the 5 phenotypic classes of carriers and deficiency allele combinations with 95% confidence intervals.

Estimation of gene frequencies. The mean frequency for the PiS allele was 24.6 (23.1–26.3) per 1000, and the mean gene frequency for PiZ was 12.7 (11.6–13.9) per 1000. Maximal gene frequencies for both PiS and PiZ are found in northern Italy, and they show a gradual decrease from north to south over the country. Very low PiS and PiZ frequencies have been found in Sardinia.

Estimation of gene prevalence. Estimates of Pi gene frequency using HWE statistics indicate that there is one carrier or deficiency-allele combination phenotype for every 13.6 individuals in the Italian population, with one in 21 of phenotype PiMS, one in 41 of phenotype PiMZ, one in 1649 of phenotype PiSS, one in 1599 of phenotype PiSZ, and one in 6201 of phenotype PiZZ.

Estimation of the numbers of carriers (PiMS and PiMZ) and deficiency-allele combinations (PiSS, PiSZ, and PiZZ). Estimates of the numbers of carriers and deficiency-allele combinations using

HWE statistics indicate a total of 4,222,365 (95% CI: 4, 218,489–4,226,245) individuals at risk, with 2,732,876 (95% CI: 2,557,746–2,919,296) of phenotype PiMS, 1,409,185 (95% CI: 1,285,010–1,544,897) of phenotype PiMZ, 34,958 (95% CI: 30,747–39,734) of phenotype PiSS, 36,051 (95% CI: 30,894–42,055) of phenotype PiSZ, and 9295 (95% CI: 7761–11,128) of phenotype PiZZ.

Portugal

Overview. A total of four control cohorts were selected from studies performed in Portugal (72–74). Results are shown in Table 4 and Fig. 3. The regions that supplied the data for the present study are located in the north, center and south of the country.

The number of individuals studied was 1488 in a total population of 10,048,232 inhabitants. Samples were collected from seamen, scholars, and cohorts of controls (HUP), not specified in detail. None of these studies was made on the general population selected at random.

Table 6. Data summaries of PiS and PiZ gene frequencies and prevalence of Pi phenotypes for carriers and deficiency-allele combinations with 95% confidence interval (CI)

Country	Total population	Gene frequency		Calculated prevalence of Pi phenotypes: 1/x (Hardy-Weinberg)					
		PiS 1000	PiZ 1000	MS	MZ	SS	SZ	ZZ	Total
France	59,329,691	69 (66-72)	10.9 (9.7-12.4)	8 (7-8)	50 (44-56)	210 (192-231)	663 (560-785)	8360 (6549-10,680)	6.52 (6.51-6.52)
Italy	57,634,327	24.8 (23-26)	12.8 (11.7-14.0)	22 (19-22)	40 (37-44)	1623 (1428-1845)	1574 (1349-1836)	6104 (5098-7130)	13.4 (13.4-13.5)
Portugal	10,048,232	86 (84-87)	12.9 (11.7-14)	5 (4-5)	43 (31-61)	60 (50-73)	288 (191-437)	5535 (2940-10,533)	3.78 (3.7-3.8)
Spain	39,996,671	109 (104-114)	9 (7.8-10.9)	5 (5-5)	61 (52-73)	84 (77-92)	496 (401-6120)	11,680 (8386-16,295)	4.49 (4.4-4.5)

Calculation of the PFS for each control cohort.

Calculated statistical values of the PFS were: mean 7.3, median 6.9, SD 3.3, and range 3.7-11.6. While high scores were calculated for two studies carried out in HUP and scholars from Lisbon (72,73), and also in HUP from all regions of Portugal (74), a study performed on seamen (75) yielded a very low score.

Estimation of gene frequencies. The mean frequency for the PiS allele was 86 (84-87) per 1000, and the mean gene frequency for PiZ was 12.9 (11.7-14) per 1000.

Estimation of gene prevalence. Estimates of Pi gene frequency using HWE statistics indicate that there is one carrier or deficiency-allele combination phenotype for every 3.8 individuals in the Portuguese population, with one in five of phenotype PiMS, one in 43 of phenotype PiMZ, one in 60 of phenotype PiSS, one in 288 of phenotype PiSZ, and one in 5535 of phenotype PiZZ.

Estimation of the numbers of carriers (PiMS and PiMZ) and deficiency-allele combinations (PiSS, PiSZ, and PiZZ). Estimates of the numbers of carriers and deficiency-allele combinations using HWE statistics indicate a total of 2,659,241 (95% CI: 2,656,601-2,661,983) individuals at risk, with 2,223,646 (95% CI: 1,990,413-2,477,409) of phenotype PiMS, 231,630 (95% CI: 165,332-

322,376) of phenotype PiMZ, 167,296 (95% CI: 138,264-201,846) of phenotype PiSS, 34,853 (95% CI: 22,969-52,531) of phenotype PiSZ, and 1815 (95%CI: 954-3418) of phenotype PiZZ.

Spain

Overview. A total of 28 control cohorts were selected from studies performed in Spain (42,76-92). The results are shown in Table 5 and Fig. 4. Most regions that supplied the data for the present study are located in the north, central, and southern parts of the Iberian Peninsula. Two populations belonging to the Balearic Islands (Majorca and Minorca), and one from the Canary Islands (Tenerife), were also included in the research. There exists a remarkable lack of data from populations of the Mediterranean coast and the west of Spain.

The number of individuals studied was 7773 for a total population of 39,966,671 inhabitants. A survey carried out in Asturias (Cantabrian Coast) (79) is the most reliable and has the highest PFS, as it was performed on a general population selected at random. The remaining Spanish surveys were carried out on seamen, blood donors, newborn infants, work-based employees, healthy controls, etc. A study performed in Majorca was carried out on a colony of Chuetas Jews, who settled on this island in the Middle Ages. Ten studies have been performed on natives from Galicia, the Basque Country, Canary Islands,

Table 7. Summaries of the estimates of the numbers of carriers and deficiency allele combinations of PiS and PiZ

Country	Total population	Calculated numbers of carriers and deficiency allele combinations (Hardy-Weinberg Equilibrium statistics)					
		MS	MZ	SS	SZ	ZZ	Total
France	59,329,691	7,748,038	1,236,256	300,517	95,899	7651	9,388,361
Italy	57,634,327	2,776,371	1,431,613	35,514	36,625	9443	4,289,566
Portugal	10,048,232	2,223,646	231,630	167,296	34,853	1815	2,659,241
Spain	39,996,671	7,824,692	659,249	494,858	83,386	3513	9,065,699
Total	167,008,921	20,572,747	3,558,748	998,185	250,763	22,422	25,402,867

and isolated populations in the Pyrenees, Central Spain and North Catalonia. As these populations are characterized by high values of PiS and low values of PiZ, this fact could have introduced bias in the global calculation of the total number of carrier and deficient Pi phenotypes from Spain, by reducing PiZ values.

Calculation of the PFS for each control cohort.

Calculated statistical values of the PFS were: mean 6.2, median 5.4, SD 1.97, and range 3.8–12.4. Twenty-two out of 28 studies have given high scores (5–12). These reliable studies were carried out in Galicia (75–77,82), Valladolid (88), Madrid (76), Catalonia (79,92), Asturias (42), the Pyrenees (85,90), Andalusia (86,91), Tenerife (87), Minorca (90), and the Basque Country (77,81,84,89). The remaining six studies yielded very low scores.

Estimation of gene frequencies. The mean frequency for the PiS allele was 109 per 1000, and the mean gene frequency for PiZ was nine per 1000. Maximal gene frequencies for PiS occur in northern Spain, and they show a slow decrease to the eastern and southern areas of the country, but in general have very high values. Values of PiZ frequencies are very erratic, with maximal values (about 20 per 1000) occurring in Asturias and Valladolid.

Estimation of gene prevalence. Hardy–Weinberg statistics suggest that there is one carrier or deficiency-allele phenotype for every 4.5 Spanish individuals; with one in 11,680 persons of the ZZ type, one in 496 of the SZ phenotype, and the remaining carriers and deficient alleles for the MS, MZ, and SS phenotypes.

Estimation of the numbers of carriers (PiMS and PiMZ) and deficiency-allele combinations (PiSS, PiSZ, and PiZZ). Estimates of the numbers of carriers and deficiency-allele combinations using HWE statistics indicate a total of 8,903,773 individuals at risk (95% CI: 8,898,617–8,908,931 with 7,691,380 (95% CI: 7,306,724–8,092,145) of phenotype PiMS, 652,603 (95% CI: 549,259–774,555) of phenotype PiMZ, 475,650 (95% CI: 434,380–520,567) of phenotype PiSS, 80,716 (95% CI 65,306–99,654) of phenotype PiSZ, and 3424 (95% CI: 2455–4769) of phenotype PiZZ.

The estimates of the numbers of carriers and deficiency-allele combinations using HWE statistics are influenced by the very different gene frequencies for PiS and PiZ on the Canary Islands, as well as on the islands of Majorca and

Minorca where the population sizes are, respectively, 930,015 (<http://www.red2000.com/spain/canarias/>), 581,564 (<http://www.info-majorca.co.uk/balearic.islands/index.php>), and 65,000 (<http://www.rtmhotels.com/rtmeng/Menorca.htm>). When the total number of residents on these three islands are subtracted from the total population of Spain, the numbers of carriers and deficiency-allele combinations are higher, with a total of 9,066,380 (95% CI: 9,061,190–9,071,571) individuals at risk, with 7,825,168 (95% CI: 7,418,973–8,248,969) of phenotype PiMS, 659,361 (95% CI: 550,922–788,198) of phenotype PiMZ, 494,929 (95% CI: 450,503–543,423) of phenotype PiSS, 83,407 (95% CI: 66,907–103,849) of phenotype PiSZ, and 3514 (95% CI: 2484–4961) of phenotype PiZZ.

A weighted-mean approach can be taken by using the data from Spain, the Canary Islands and the islands of Majorca and Minorca, by adjusting the data for the percentages of these four populations in the total population of Spain. This exercise provides the numbers of carriers and deficiency-allele combinations as follows: a total of 9,065,699 (95% CI: 9,060,509–9,070,890) individuals at risk, with 7,824,692 (95% CI: 7,774,710–7,872,756) of phenotype PiMS, 659,249 (95% CI: 655,038–663,298) of phenotype PiMZ, 494,858 (95% CI: 494,858–544,364) of phenotype PiSS, 83,386 (95% CI: 66,590–104,285) of phenotype PiSZ, and 3513 (95% CI: 2466–4995) of phenotype PiZZ.

This type of analysis demonstrates that when it is possible to obtain estimates of the size of subgroup populations, the weighted-mean approach provides the most accurate estimates of the numbers of carriers and deficiency-allele combinations.

Discussion

Overview

A total of 92 cohorts with a total cohort sample size of 38,682 individuals from France, Italy, Portugal and Spain, were used to calculate gene frequencies, gene prevalence (Table 6), and also to estimate the numbers of individuals with each of the five phenotypes of AAT-deficiency carriers and deficiency-allele combinations for PiS and PiZ at risk for adverse health effects (Table 7). This tabulation demonstrates very striking differences among the gene prevalences of carriers and deficiency-allele combinations for PiS and PiZ within this part of southern Europe. Both the PiS and PiZ alleles are found in all four countries, with the lowest frequency of PiZ

observed in Italy. These data confirm and extend the original observations of Hutchison (47), and those of Blanco (43–45).

With an estimated total population of 167,008,921 individuals for these four countries, the total population at risk consists of 25,402,867 carriers and deficiency-allele combinations for PiS and PiZ. The authors of the present survey are aware that these data should be considered an approximation, as our calculations might have bias related to: the source of the subjects selected; sample size; and laboratory technique and diagnosis (93). However, it is our intention to provide these numbers with 95% CI to illustrate: the very large numbers of individuals at risk in these four countries, and the need for follow-up epidemiological studies to confirm and extend these original observations.

Comparison of the control cohort data taken from various genetic epidemiological studies

A wide range of subjects have been chosen for study in various southern European surveys, including: blood donors; seamen; subjects recruited from a population register; university students; newborn infants; healthy controls; work-based employees, natives, etc. In some studies, subjects recruited are poorly described as 'randomly selected' or 'unrelated persons', although selection procedures are not often given in detail. Natives are commonly defined as subjects with four grandparents born in a specific area, although some authors do not clearly specify the significance of the term 'native'. Surveys representative of a general population (i.e. performed on randomly selected subjects of all ages, obtained from a municipal population register) provide the best data on the actual gene frequencies of PiS and PiZ, as illustrated in the study of Blanco et al. (43).

Potential sources of bias

As ideally a sample should be representative of the general population in every country, some imprecision could be attributed to sample selection in the present report. Several studies have been carried out in highly selected populations (i.e. 'natives' from Galicia, the Basque Country, Normandy, Brittany, the Canary Islands, Chuetas Jews from Majorca, etc.). These populations have great ethnographic interest, but they are not representative of the general population of any specific region or country, as they are in a minority. In these cases, we have used a weighted-

mean approach that takes into account the size of these populations in relation to the total population of a given country. This approach is not always possible when the control cohort is poorly defined and estimates of the population size of a subgroup cannot be determined.

In the present study, the database for some regions was limited both in the number of cohorts and in their size; thus, caution must be exercised with regard to the values of the PiS and PiZ alleles in these areas.

Development and use of the PFS to assess the scientific quality of individual control cohorts

Differences in sample size and 95% CI have been taken into account by means of PFS that were generated to assess the scientific quality of each survey. This approach provides an objective assessment of each survey in a given country. It is important to bear in mind that many of the control cohort data used in the present study were not developed as detailed genetic epidemiological studies. Many were developed to compare normal subjects to patients with AAT deficiency and various health problems; they were not intended as surveys of the general population or a given ethnic subgroup in a given city, or geographical region of a given country. In addition, we have not established a cut-off value for selecting studies, as we consider our work informative instead of selective. However, in our attempts to develop criteria for new studies, our analysis of present control cohort data suggests that a survey should have a score of at least 5 points. Based on this criterion, data reported from five out of 20 surveys in France, 12 out of 40 in Italy, one out of four in Portugal, and six out of 28 in Spain, should be considered with caution.

The role of laboratory errors

Some miscalculations might be attributed to laboratory errors, but these cannot be detected in the present analysis. Some errors might be related to technical issues, such as sample storage. For example, it is recognized that a misdiagnosis is possible if storage conditions are not appropriate, as bands become discolored. Besides, a false FM pattern generated from M bands in aged samples has been reported (47). On the other hand, a false SZ pattern might appear in ZZ individuals with liver disease (47). Interpretation of an IEF pattern demands experience and skill. A typical error from inexperienced persons is to confuse M2 and Z bands (42). Occasionally, we have found errors in the calculations of allele frequency.

The method used for characterization of phenotypes is also important. In the present analysis, only studies carried out by crossed antigen-antibody electrophoresis or IEF have been selected, as both are considered to be reliable techniques. In addition, the technique of starch-gel electrophoresis (SG) is considered obsolete because it underestimates the Z-gene frequency as a result of the difficulty in distinguishing Z bands from the MZ heterozygote band. However, none of the studies in the present analysis reported using IEF followed by polymerase chain reaction (PCR) for validation of abnormal or inconclusive findings of IEF.

Conclusions

In spite of the above-mentioned limitations, the development of the present database demonstrates that there are a large number of individuals at risk for adverse health effects related to AAT deficiency in France, Italy, Portugal, and Spain. There are many geographical areas in each of these four countries where there is a total lack of genetic epidemiological data, thus providing an opportunity for the development of studies in the general population in those unexplored regions.

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