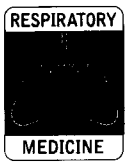
RESPIRATORY MEDICINE (1998) **92**, 367–377



Topical Reviews

α_1-Antitrypsin deficiency in Europe: geographical distribution of Pi types S and Z

D. C. S. HUTCHISON

Department of Respiratory Medicine, King's College School of Medicine, London, U.K.

α_1-Antitrypsin (AT) is the principal serum inhibitor of proteolytic enzymes such as neutrophil elastase. AT can exist as over 90 different genetically determined variants known as the Pi system; the three most important variants are type M (90% of population) and types S and Z, two of the commoner abnormal variants. Homozygotes of type Z have a severe reduction in the serum AT concentration and may develop pulmonary emphysema or hepatic cirrhosis. Heterozygotes of type SZ have a less severe reduction in serum AT concentration and the association with clinical disease is less clear.

The S and Z variants are found mainly among those of European stock. The gene frequency for Pi type Z is highest on the north-western seaboard of the continent and the mutation seems likely to have arisen in southern Scandinavia. The distribution of type S is quite different; the gene frequency is highest in the Iberian peninsula and the mutation is likely to have arisen in that region. A population survey for determining the number of type Z homozygotes in a given community is important for planning purposes now that AT replacement therapy is potentially available.

RESPIR. MED. (1998) **92**, 367–377

Introduction

α_1-Antitrypsin (AT) deficiency is a genetic disorder predominantly arising in those of European stock. AT is the main serum inhibitor of proteolytic enzymes and it is believed that, in AT deficiency, enzymes such as neutrophil elastase can damage the lung tissues leading to the disabling syndrome of pulmonary emphysema. The original cases were recognized by Swedish workers (1,2) and their description of the clinical features has been confirmed on many occasions (3–5). An association between AT deficiency and neonatal hepatitis was first described by Sharp *et al.* (6) and there is a risk of cirrhosis in adult life (7). This is thought to be due to the presence of inclusion bodies of AT deposited within the hepatocytes.

The term AT was introduced by the original discoverers of the condition, but it is now recognized that the main function of the protein is the inhibition of neutrophil elastase. Terms such as α_1-proteinase inhibitor or α_1-antiproteinase have been introduced as a more accurate functional description but the more familiar name AT will be used in this article.

AT deficiency has been reported in virtually every country in Europe but it now seems to have been no accident that the initial discovery was made in Sweden; Eriksson in a recent historical review of this period (8) has reminded us of the long Swedish tradition of research in protein chemistry and particularly in protein separation techniques. The method of paper electrophoresis was much improved by Laurell; the absence of the protein band in the α_1 region of type Z homozygotes was first detected by this method (1) and shown to be associated with pulmonary emphysema.

Correspondence should be addressed to: D. C. S. Hutchison, Department of Respiratory Medicine, King's College School of Medicine, Denmark Hill, London SE5, U.K.

Methods

New advances in the methodology soon indicated that AT is a polymorphic system with a large number of possible biochemical variants (the Pi system). This was first demonstrated independently, again in Scandinavia, by two groups of workers (9,10) using the method of starch gel electrophoresis (SG). A problem with this otherwise very useful technique was the difficulty in recognizing the Z bands due to the small quantity of AT actually reaching the serum. This particularly affected the recognition of the MZ heterozygote where the M bands could appear normal while the Z bands went undetected. With the homozygous deficiency (Pi type ZZ) the difficulty did not usually arise as one could readily deduce the probable phenotype from the total absence of M bands and if necessary this could be confirmed by direct measurement of the serum AT

0954-6111/98/030367+11 $12.00/0

concentration by an immunological method. The rarity of the Z homozygote means, however, that gene frequencies must be calculated almost entirely from the frequency of the MZ heterozygote which may be significantly underestimated by the SG method.

To overcome this problem the method of 'crossed antigen–antibody electrophoresis' (XAA) was introduced (11). The individual starch gel is cut out and run again at right angles into the medium containing the antibody. Prevalence studies in which SG was used alone (without XAA) have generally given lower frequencies for Pi type Z than those which include XAA; the combined method has usually given results consistent with modern techniques. XAA was time consuming and expensive and has been replaced by the technique of isoelectric focusing in polyacrylamide gels (IEF) (12–14).

The bands associated with Pi type S are usually much more readily detectable than those of type Z even with SG alone and the gene frequency results obtained by the various techniques are more consistent. Since the introduction of IEF, over 90 variants have been recognized (15), although most are very rare and few are of clinical importance. There are five or more subtypes of the common M variant which have allowed the method to be widely used in parentage testing and in forensic investigations.

The conditions of sample storage are also of importance and it has been observed that the specific bands may be seriously weakened if the specimens are subjected to repeated freezing and thawing. A false FM pattern can be generated from type M in aged samples or if storage conditions have been unsatisfactory (16). In liver disease, a false SZ pattern may be seen in homozygotes of type Z (17). For further details of the methodology the reader is referred to more detailed reviews (16,18).

DRIED BLOOD SPOT METHOD

Dried blood samples collected on filter paper have been used as a convenient method of screening newborn infants (19). After elution, the sample phenotypes are assessed by IEF. Again, the storage conditions are important if valid results are to be obtained.

Alternatively, the serum AT concentration can be obtained from the eluate by an immunological method (20). It is then necessary to choose a cut-off value below which the majority of severely deficient sera (e.g. type ZZ) are likely to fall. A fresh blood sample is then obtained for those falling into the deficient range. By this method few of the MS or MZ heterozygotes will be detected and gene frequencies therefore cannot be calculated.

DNA-BASED ASSAYS

Methods of DNA analysis utilizing the polymerase chain reaction have been developed which allow detection of the S and Z mutations (21,22). These methods have not yet been reported in population surveys.

Prevalence Surveys

Almost all prevalence surveys in Europe since about 1976 have been performed by the method of IEF and the available results, together with some earlier results obtained by SG and XAA (but not reports of type Z obtained by SG alone), are included in Table 1. Certain laboratories have introduced their own variations on the IEF technique depending on the precise purpose of the survey; for instance, a method which is optimal for separating the M subtypes may be less than optimal for detecting the more slowly moving variants such as types S and Z. These methods will not be discussed in detail here.

Patients homozygous for Pi type Z are the most commonly encountered form of severe AT deficiency associated with emphysema. One purpose of a population survey is to detect Pi ZZ patients at risk of developing emphysema and to determine their total number in a given community. This is particularly important as specific replacement therapy is potentially available (83) although its effectiveness is not yet known.

Gene Frequency

The prevalence of the various gene types in any population is best expressed as the 'gene frequency' which can be defined as the frequency of all genes of a particular type whether occurring in homozygotes or heterozygotes. Thus type MZ individuals contribute one Z gene to the total whereas type ZZ individuals contribute two. It can be shown by the 'Hardy–Weinberg' principle (84) that the prevalence of the type Z homozygote is equal to the square of the gene frequency for that allele. For example, in Southern Sweden (Table 1), where the gene frequency for type Z is quoted as 0·0231 or 23·1 per 1000, the calculated ZZ homozygote prevalence is 0·00053 or 1 in 1870. By contrast, in Northern Sweden, the gene frequency for type Z is 8·3 per 1000 and the calculated ZZ homozygote prevalence is therefore only 1 in 14 500.

The gene frequency for type Z is determined almost entirely by the prevalence of the heterozygous subjects, mainly MZ, who carry over 95% of the type Z genetic material. Any error in the gene frequency estimate will therefore be greatly magnified when the prevalence of the homozygote is calculated. Pi type S is also a deficient phenotype especially when combined in the heterozygous form SZ where the serum AT concentration is usually 30–40% of normal. Even so, this phenotype appears to predispose to emphysema only in smokers (85). Type S is the commonest of the non-M variants and it is clearly important to assess its frequency in any survey. The prevalence of the SZ phenotype can also be calculated from the Hardy–Weinburg principle, as twice the product of the respective gene frequencies for types S and Z; again, errors in the gene frequency estimates will be much magnified in this calculation.

TABLE 1. Gene frequencies in Europe

Country	Subjects		Gene frequency*		Method	Reference
	No.	Source	S	Z		
Northern Europe						
Norway						
Oslo & Stavanger	390	BD	17·9	–	SG	(10)
Oslo	2830	BD PG	23·0	15·7	XAA	(23)
	400	BD Pat	30·0	18·8	IEF	(24)
Sweden						
South	1062	Pat	24·5	23·1	IEF	(25)
North	1869	Army	10·4	8·3	IEF	(26)
Umea (North)	619	Pop	12·1	10·5	IEF	(27)
Finland						
Non-Lapps	223	Pop Lab	0	4·5	XAA	(28)
Helsinki	136	Pop	0	10·0	IEF	(29)
Helsinki	548	BD	17·3	13·7	IEF	(30)
	300	Army	15·0	13·0	IEF	(26)
Oulu	200	NB	17·5	5·0	IEF	(31)
Lapps						
Finnish	468	Pop	3·2	1·1	XAA	(28)
Norwegian	302	Pop	0	8·3	XAA	(28)
Swedish	217	Stud	4·6	0	IEF	(26)
Denmark	909	Pat PG	22·0	22·6	IEF	(32)
Iceland	94	Pop	10·6	0	XAA	(33)
Western Europe						
Netherlands						
Industrial town	672	Pop	24·6	17·9	IEF	(34)
NE rural	802	Pop	8·8	7·4	IEF	(34)
	708	BD	29·7	4·9	IEF	(35)
	131	Lab PG	42	27	IEF	(29)
	357	Pop	29·4	12·6	IEF	(36)
United Kingdom						
N Ireland	1995	Workers	42·4	20·8	XAA	(37)
SW England	926	BD	47·5	22·1	IEF	(38)
London	2310	Pat	46·3	18·2	IEF	(39)
England	3174	Bl samp	45·5	–	SG	(40)
Scotland	1291	Bl samp	45·7	–	SG	(40)
NW England	316	BD Pat Pop	52·2	9·5	IEF	(41)
France						
Normandy	394	BD	66	22	XAA	(42)
Normandy	1030	BD	63·1	17·9	XAA	(43)
Brittany						
(Morbihan)	280	BD	75·0	23·2	XAA	(44)
(Bigoudens)	397	Pop	63·0	16·4	IEF	(45)
(Non-Bigoud)	100	Pop	60·0	5·0	IEF	(45)
Lyon	1653	BD	71·3	14·2	IEF	(46)
East						
Nancy	1551	Workers	51·9	11·0	IEF	(47)
North						
Rouen	419	Workers	68·0	7·2	IEF	(47)
Paris-Clichy	371	Workers	90·3	6·7	IEF	(47)
Paris-Creteil	151	Workers	76·2	13·2	IEF	(47)
Paris-Villejuif	719	Workers	84·1	11·1	IEF	(47)
South-west						
Toulouse	1247	Workers	105·0	12·8	IEF	(47)
Bordeaux	356	Workers	125·0	22·5	IEF	(47)
South						
Marseille	239	Workers	37·6	4·2	IEF	(47)

TABLE 1. Continued

Country	Subjects		Gene frequency*		Method	Reference
	No.	Source	S	Z		
Western Europe						
Belgium	1345	NB	54·3	16·7	IEF	(48)
Spain						
Madrid	378	Seamen	112	11·9	XAA	(49)
Madrid	103	Hosp	82·5	0	IEF	(50)
Seville	170	Pop	97·1	0	XAA	(51)
Galicia	131	Pop	99·3	11·4	XAA	(51)
Galicia	129	Pop	147	3·9	XAA	(51)
Basque region	480	Pop	149	9·4	IEF	(52)
Native (Arratia)	146	Pop	116	6·9	XAA	(51)
Native	56	BD	107	0	IEF	(53)
Other Basque	167	Pop	99	0	IEF	(54)
Other Basque	810	BD	116	7	IEF	(53)
	176	Pop	88	17	IEF	(54)
Portugal						
Lisbon	39	Seamen	141	0	XAA	(49)
Lisbon	330	Pop	115·2	18·2	XAA	(55)
All regions	219	Stud	54·8	22·8	XAA	(56)
	900	Pop	152	10	IEF	(57)
Central Europe						
Germany						
Hamburg	516	Pop	21·3	8·7	XAA	(33)
Marburg	262	Pop	22·9	9·5	XAA	(58)
Munich	146	Pat	34·2	13·7	IEF	(59)
Munich	538	BD Lab	22·3	12·1	IEF	(60)
Hessen	280	Workers	30	14	IEF	(61)
South	229	BD	24·0	10·9	IEF	(62)
South	357	Pat	23·0	18·7	IEF	(63)
	752	Pat	17·3	12·7	IEF	(64)
Austria						
Tyrol	868	BD	22·5	13·8	IEF	(65)
Switzerland						
Zurich	1148	Pat	38·3	11·3	IEF	(66)
Southern Europe						
Italy						
S Tyrol						
Italian	1606	NB	31·8	15·0	IEF	(67)
German	7522	NB	14·6	19·5	IEF	(67)
Turin	394	BD NB	31·8	8·3	IEF	(68)
Cuneo	208	BD NB	55·0	4·7	IEF	(68)
Parma	268	BD NB	24·7	13·3	IEF	(68)
Verona	202	Hosp	29·7	9·9	IEF	(69)
Bologna	263	BD NB	14·0	8·5	IEF	(68)
Genoa	389	BD	33·9	10·2	IEF	(70)
Livorno	490	BD NB	29·6	7·1	IEF	(68)
Grosseto	172	BD NB	17·8	2·9	IEF	(68)
Arrezzo	472	NB	36·0	5·2	IEF	(70)
Perugia	100	BD NB	27·4	0	IEF	(68)
Spoleto	120	BD NB	42·7	8·5	IEF	(68)
Rome	500	NB	67·0	15·0	IEF	(71)
Rome	967	NB	43·9	10·8	IEF	(70)
Rome	513	BD	28·3	9·7	IEF	(72)
Campobasso	600	BD NB	26·9	3·0	IEF	(68)
Naples	260	NB	25·0	11·6	IEF	(73)

TABLE 1. Continued

Country	Subjects		Gene frequency*		Method	Reference
	No.	Source	S	Z		
Southern Europe						
Italy						
Sicily						
Palermo	175	BD NB	16·3	8·1	IEF	(68)
Sardinia						
Cagliari	100	BD NB	62·5	0	IEF	(68)
Cagliari	218	BD	61·9	4·6	IEF	(74)
Serbia	1060	BD	6·6	12·7	IEF	(75)
Greece						
Nine regions	400	Pop	2·5	16·2	XAA	(33)
Athens	504	Pop	28	2	XAA	(76)
	160	Hosp	3·1	6·2	IEF	(77)
Eastern Europe						
Hungary						
Twelve groups	172	Pop	17·4	14·5	XAA	(33)
	1282	Pop	14·0	6·0	IEF	(78)
Poland						
Poznan	3560	BD	15·6	–	SG	(79)
	631	BD Pop	14·2	15·0	IEF	(80)
Russia						
Moscow	402	Pop	6·2	4·8	IEF	(81)
Kalinin	1049	Pop	16·5	3·2	IEF	(82)

BD, blood donors; NB, newborns; Pat, parentage tests; PG, pregnant women; Lab, laboratory or hospital staff; Stud, school or college students; Hosp, hospital patients; Bl samp, blood samples; Pop, population register, electoral roll, randomly selected, healthy or unrelated subjects; Workers, employees or workplace survey; SG, starch gel; XAA, crossed antigen–antibody electrophoresis; IEF, isoelectric focusing. *Gene frequency: per 1000.

SAMPLE SIZE

The sample size in the various surveys (Table 1) ranges from 39 to 7522 subjects, although in over half of the surveys the number of subjects lies between 100 and 500. The total numbers available in small isolated communities (which are potentially of great interest) may be quite limited, increasing the possibility of error in the estimate of gene frequency.

The 95% confidence limits for three allele population sizes at three typical gene frequencies are given in Table 2. The confidence limits are wide especially when gene frequency is low, and it follows that, in any population sample, the number of subjects (half the number of alleles) should ideally be 250 or more.

SELECTION OF SUBJECTS

A wide range of subjects have been chosen for study in the various surveys. They include blood donors, army conscripts, seamen, laboratory or hospital staff, pregnant women, work-based employees, students, newborn infants, subjects involved in parentage disputes and subjects obtained from a population register or electoral roll; in some surveys, the subjects are described as 'randomly selected' or 'unrelated', although the details of the selection procedures are often not given in detail.

The S and Z Phenotypes Analysed by Country and Region

All European population surveys known to the author have been quoted (Table 1) provided that the subjects appear to represent a randomly selected 'normal' group and that Pi type was determined in all subjects by IEF or by XAA. In surveys where SG was used alone (as in many of the early studies) the gene frequencies for type Z have been omitted from Table 1, as they may be underestimated by this method. Surveys in which subjects with abnormal phenotypes such as SZ or ZZ were identified only after an initial screening procedure have also been omitted as true gene frequencies cannot be calculated under these circumstances. The district or origin of the subjects is given if specified by the authors.

TABLE 2. Confidence limits (95%) at various gene frequencies and population sizes

Gene frequency	No. of alleles	95% confidence limits
100	1000	82–120
	500	75–130
	200	62–150
20	1000	12–31
	500	10–37
	200	5–50
10	1000	5–18
	500	3–23
	200	1–36

Gene frequencies and confidence limits (86) expressed per 1000 alleles.

If the gene frequencies are not given by the authors, they have been calculated from the quoted phenotype frequencies. The gene frequency is obtained in the standard manner by summing the total number of alleles in question (Pi type Z, for instance) and expressing this number as a fraction of the total number of Pi type alleles in the population; the total number of alleles is of course twice the number of subjects. For ease of comparison, all gene frequencies have been expressed per 1000 genes; thus, in the first line of Table 1, the gene frequency for Pi type S in Norway is given as 17·9 per 1000 instead of the conventional 0·0179.

Distribution of Gene Frequencies for Pi Types S and Z (Fig. 1)

PI TYPE S

The distribution of the type S gene within Europe is shown in Fig. 1(a). The value declines from east to west over the whole continent, ranging from <20 per 1000 in the east to >90 in the Iberian peninsula. A similar high figure is found among the Basque community although they are regarded as an unusual population from the genetic point of view as discussed below. The high gene frequency suggests that the S mutation may have arisen in the north of the Iberian peninsula in prehistoric times and spread eastwards by large-scale population movements, the process known as 'gene flow'. A very low type S frequency on the other hand is seen in the isolated Lapp community.

The distribution of the deficient heterozygous phenotype SZ is dominated by the high gene frequency for type S and shows a similar decrease from west to east. The highest calculated figure is obtained in Portugal (about 1 in 330) falling to about 1 in 5000 in eastern Europe.

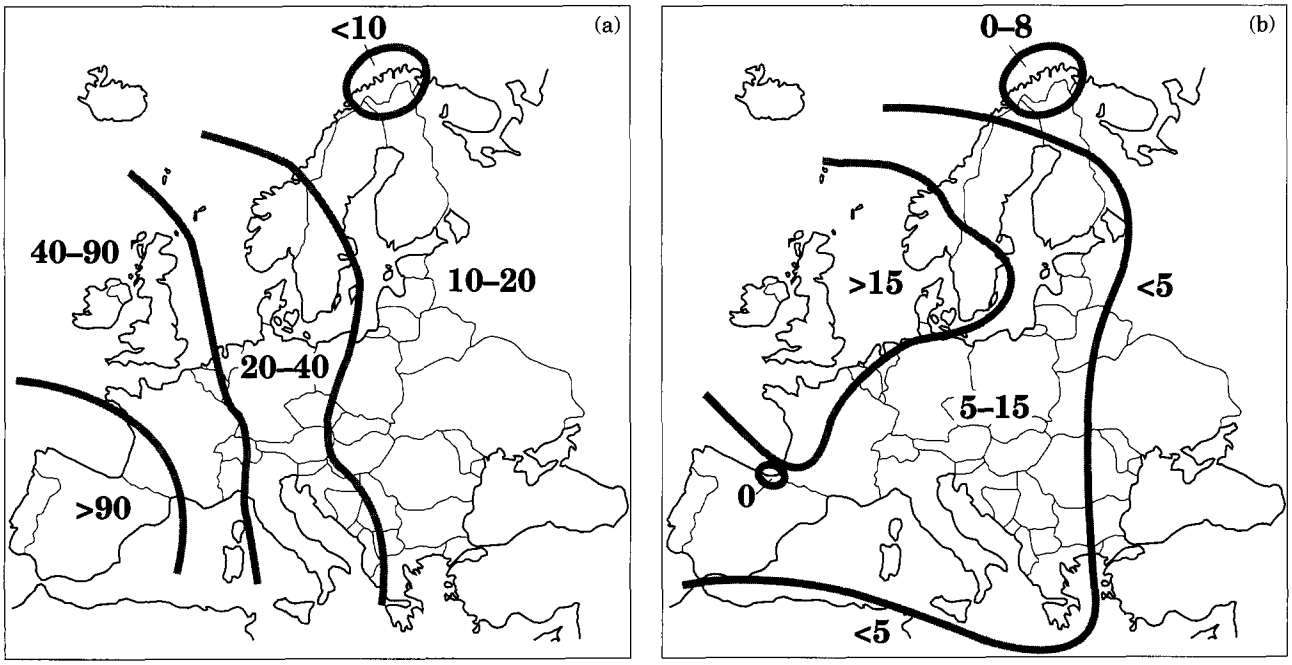


FIG. 1. Distribution (a) of Pi type S and (b) of Pi type Z in Europe. The gene frequency is expressed as the total number of Z or S genes (whether in homo- or heterozygotes) per 1000 genes of all Pi types. Shaded lines: 'isogenes' or lines of equal gene frequency (drawn by eye). The distribution of the two genes differs markedly; the greatest frequency of type Z is on the north-western seaboard of the continent whereas that of type S is greatest in the Iberian peninsula. Considering the isogenes for type Z, the gene frequencies of 15 per 1000 and 5 per 1000 are equivalent to homozygous ZZ prevalences of, respectively, 1 in 4440 and 1 in 40 000. (Political boundaries are inserted for guidance only.) Reproduced (with modification) from *Respiratory Medicine* 2nd edition (Eds Brewis *et al.*) 1995. By courtesy of W. B. Saunders Co. Ltd.

PI TYPE Z

The distribution of gene frequency for type Z [Fig. 1(b)] differs markedly from that of type S. The frequency of type Z in most countries is much less than that of type S so the data are rather less firm but the striking feature of the map is the relatively high gene frequency on the north-western seaboard of the continent. The gene frequency is very low among the Lapp and Basque communities. As with type S, the gene frequency is lower in the eastern regions of the continent and from other surveys (87) both types are rare among non-Europeans. It has been suggested (88) that the Z mutation occurred 66 generations or about 2000 years ago. The most likely site of origin seems to have been southern Scandinavia from the high gene frequency in that area and the known migration patterns, exemplified by the Viking colonization of north-western Europe in the 9th to 11th centuries AD.

Special Points about Various Regions

SCANDINAVIA AND THE LAPPS

The first study of AT phenotype frequency in any part of the world was reported from Norway using the newly developed technique of starch gel electrophoresis (10) and Norwegian and Finnish Lapps were studied by this method at an early stage (28). The Lapps are a population of great interest because of their evident isolation from other genetic influences. The gene frequencies for types S and Z in Finnish and Swedish Lapps are among the lowest recorded in Europe (26,28) although the figure for type Z (28) obtained in Norwegian Lapps was rather greater than among the other groups.

There has been some speculation about the low prevalence of type Z in this region; by one theory, genes predisposing to pulmonary disease could have been eliminated by the extreme climate of the northern winter. This theory assumes that heterozygotes for both Pi types S and Z have a similar susceptibility. The results appear more likely to be due to the fact that these culturally and linguistically separate peoples have remained as an 'isolate' and have never intermarried to any significant extent with those population groups bearing the S and Z mutations.

Evidence from the distribution of the ABO blood groups among Lapps supports the latter viewpoint. The frequency of the A gene among Lapps and particularly of the sub-type A2 is much higher than in neighbouring regions (89) and the frequencies of many other blood group systems are also unusual; these authors observe that 'in nearly all gene frequencies the Lapps differ widely from all other European populations' and that this seems to be due to their long isolation as a small population. Analysis of genetic divergence from other European populations (based on 88 genes) indicates that the Lapps are 'extreme outliers' followed in order by the Sardinians, Basques and Icelanders (90).

The Swedish study of Sveger (91) in 200 000 newborn infants is the only survey large enough to enable a direct estimate of the prevalence of the ZZ homozygote to be obtained. One hundred and twenty type ZZ infants were detected giving a prevalence of 0·0006 or 1 in 1670 which is very close to the figure calculated from the gene frequency in the southern part of the country (25).

ICELAND

There has been only one survey (by XAA) among the Icelandic population (33). The gene frequency for type Z was found to be zero although the sample was small, consisting of only 94 subjects. In this study, however, the prevalence of Pi type F now appears to have been considerably overestimated (gene frequency: 117 per 1000) possibly because of overlong storage which can generate false FM patterns from type M (16,40). Errors in estimates of the prevalence of types S and Z therefore seem possible. Historical records indicate that the original colonists emigrated into Iceland during the 11th century mainly from Norway with a smaller group originating in Ireland. A survey of ABO blood groups, however, indicates a closer affinity to Ireland than to Norway (89). It would therefore be interesting to see the results of a survey by modern methods in this population.

NETHERLANDS

The results of surveys within a given country may differ considerably (34). In the industrial town of Vlaardingen, close to the great seaport of Rotterdam, the gene frequencies for both S and Z were similar to those of other nearby industrial areas. The same authors surveyed the small rural town of Vlagtwedde in the north-east of the country, with a population which 'hardly ever moves outside of its birthplace'; they found very much lower gene frequencies for both alleles, suggesting a community relatively isolated from large population movements.

FRANCE

A large population of over 5000 employees in eight separate regions was studied by IEF as part of an epidemiological survey (47). The data together with those of other surveys indicate that the gene frequency for type S is substantially higher in south-western France and approaches that found in northern Spain. In the far south (Marseille), the gene frequencies for both types S and Z (47) were lower than in any other region of the country, possibly as a result of immigration into the area from Africa or Asia.

The inhabitants of the Bigouden region in the west of Brittany have specific sociological patterns and have been the subject of special study (45); the gene frequencies for S and Z, however, do not differ from those of other Bretons.

SPAIN, PORTUGAL AND THE BASQUE REGION

A very high gene frequency for type S was noticed in an early study (49) among Spanish seamen calling at Norwegian ports. Some of the highest figures for type S in

Europe have been obtained in Portugal and in Galicia in north-western Spain; it seems likely that the S mutation occurred in this region at an unknown epoch.

The Basque Region

The inhabitants of the Basque region have aroused interest from the genetic point of view as they are considered to be a group who have largely remained isolated from their immediate neighbours until recent times (54). Their language, Euskera, is the only one spoken in western Europe, apart from the Lapp tongue, which does not belong to the Indo-European family. They have the lowest frequency for blood group B in Europe, 0–3% in various surveys (51,89), and the highest for Rhesus group d (89). It has been suggested (90) that the Basques are the descendants of the late paleolithic inhabitants of western Europe who were displaced by the neolithic peoples with high blood group B frequencies moving from the east.

In two studies (53,54), the Pi types of subjects considered to be of pure Basque descent were examined. The gene frequencies for type S were at much the same high levels as found in other parts of Spain. For type Z, the gene frequency was found to be virtually zero in the pure Basque subjects. The gene frequency for type Z in Madrid was also found to be zero and is low in other regions of northern Spain. In spite of other marked genetic differences, the Pi type frequencies do not, in themselves, demonstrate any obvious differences between the Basques and their immediate neighbours.

ITALY

A large number of surveys have been reported from many regions and cities in Italy. Seventeen are quoted in one review (68) and a selection of these is given in Table 1. There is a wide range of frequencies particularly for type S. Weighted averages of the 17 surveys from mainland Italy (excluding the German Tyrol) with a total of 7524 subjects give gene frequencies of 34·6 per 1000 for type S and 10·0 per 1000 for type Z. In a large survey of over 9000 subjects in the South Tyrol, there was an obvious difference between Italian and German speakers in the gene frequency for type S (67).

In Sardinia, for subjects confirmed as being of local origin, the gene frequency for type S is much greater, and for type Z much smaller, than in most other regions of Italy (68,74). When other polymorphic systems are examined (blood groups or HLA types, for instance), Sardinia emerges as genetically very far removed from all other Italian regions, including Sicily (90,92); the frequency of Rhesus group d is almost the lowest in Europe (89).

GREECE

Three studies (33,76,77) are available, the results of which differ considerably. Only the latest study was performed with IEF, giving a very low frequency for type S and an intermediate frequency for type Z. In terms of genetic divergence, the Greeks are regarded as among the 'outliers', although less extreme than the Lapps and Basques (90). A larger study of the Pi system among the Greeks would be of interest.

HUNGARY

In the more recent study (78), gene frequencies were obtained in 1282 subjects divided into 12 population groups. The overall gene frequencies are given in Table 1. Certain population groups had a significantly lower gene frequency for type S, firstly the Gipsies, thought to originate in the Indian subcontinent, and secondly the Jász people, perhaps originating in China.

References

1. Laurell CB, Eriksson S. The electrophoretic alpha1-globulin pattern of serum in alpha-1-antitrypsin deficiency. *Scand J Clin Invest* 1963; **15:** 132–140.
2. Eriksson S. Studies in alpha-1-antitrypsin deficiency. *Acta Med Scand* 1965; **177** (Suppl. 432): 1–85.
3. Kueppers F, Black LF. Alpha-1-antitrypsin and its deficiency. *Am Rev Respir Dis* 1974; **110:** 176–194.
4. Tobin MJ, Cook PJL, Hutchison DCS. Alpha-1-antitrypsin deficiency: the clinical and physiological features of pulmonary emphysema in subjects homozygous for Pi type Z: a survey by the British Thoracic Association. *Br J Dis Chest* 1983; **77:** 14–27.
5. Brantly ML, Paul LD, Miller BH, Falk RT, Wu M, Crystal RG. Clinical features and history of the destructive lung disease associated with alpha-1-antitrypsin deficiency of adults with pulmonary symptoms. *Am Rev Respir Dis* 1988; **138:** 327–336.
6. Sharp HL, Bridges RA, Krivit W, Freier EF. Cirrhosis associated with alpha-1-antitrypsin deficiency: a previously unrecognised inherited disorder. *J Lab Clin Med* 1969; **73:** 934–939.
7. Eriksson S, Carlson J, Velez R. Risk of cirrhosis and primary liver cancer in alpha-1-antitrypsin deficiency. *N Engl J Med* 1986; **314:** 736–739.
8. Eriksson S. Alpha-1-antitrypsin deficiency: lessons learned from the bedside to the gene and back again. Historic perspectives. *Chest* 1989; **95:** 181–189.
9. Axelsson U, Laurell CB. Hereditary variants of serum alpha-1-antitrypsin. *Am J Hum Genet* 1965; **17:** 466–472.
10. Fagerhol MK, Braend M. Serum prealbumin: polymorphism in man. *Science* 1965; **149:** 986–987.
11. Fagerhol MK, Laurell CB. The polymorphism of 'prealbumins' and alpha-1-antitrypsin in human sera. *Clin Chim Acta* 1967; **16:** 199–203.
12. Arnaud P, Chapuis-Cellier C, Creyssel R. Polymorphism of plasma alpha-1-antitrypsin (Pi system). Demonstration by electrofocusing on polyacrylamide gel. *C R Seances Soc Biol Ses Fil* 1974; **168:** 58–64.

13. Allen RC, Harley RA, Talamo RC. A new method for determination of alpha-1-antitrypsin phenotypes using isoelectric focusing on polyacrylamide gel slabs. *Am J Clin Pathol* 1974; **62:** 732–739.
14. Frants RR, Eriksson AW. Alpha-1-antitrypsin: common subtypes of Pi M. *Hum Hered* 1976; **26:** 435–440.
15. Faber JP, Poller W, Weidinger S, Kirchgesser M, Schwaab R, Bidlingmaier F, Olek K. Identification and DNA sequence analysis of 15 new alpha-1-antitrypsin variants, including two PI*QO alleles and one deficient PI*M allele. *Am J Hum Genet* 1994; **55:** 1113–1121.
16. Fagerhol MK, Cox DW. The Pi polymorphism: genetic, biochemical, and clinical aspects of human alpha-1-antitrypsin. *Adv Hum Genet* 1981; **11:** 1–62.
17. Whitehouse DB, Lovegrove JU, Mieli-Vergani G, Mowat AP, Hopkinson DA. 'SZ like' alpha-1-antitrypsin phenotypes in PI ZZ children with liver disease. *Ann Hum Genet* 1994; **58:** 11–17.
18. Norman MR, Mowat AP, Hutchison DCS. Molecular basis, clinical consequences and diagnosis of alpha-1-antitrypsin deficiency. *Ann Clin Biochem* 1997; 34: 230–246.
19. Jeppsson JO, Sveger T. Typing of genetic variants of alpha-1-antitrypsin from dried blood. *Scand J Clin Lab Invest* 1984; **44:** 413–415.
20. Schoos R, Dodinval-Versie J, Verloes A, Lambotte C, Koulischer L. Enzyme immunoassay screening of alpha-1-antitrypsin in dried blood spots from 39 289 newborns. *Clin Chem* 1991; **37:** 821–825.
21. Newton CR, Graham A, Heptinstall LE, Powell SJ, Summers C, Kalsheker N, Smith JC, Markham AF. Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). *Nucleic Acids Res* 1989; **17:** 2503–2516.
22. Bruun-Petersen K, Bruun-Petersen G, Dahl R *et al.* Alpha-1-antitrypsin alleles in patients with pulmonary emphysema, detected by DNA amplification (PCR) and oligonucleotide probes. *Eur Respir J* 1992; **5:** 531–537.
23. Fagerhol MK. Serum Pi types in Norwegians. *Acta Path Microbiol Scand* 1967; **70:** 421–428.
24. Moller P, Fagerhol MK. Pi phenotypes in patients with ankylosing spondylitis, acute anterior uveitis, psoriasis and chronic prostatitis. *Clin Genet* 1984; **26:** 339–341.
25. Hjalmarsson K. Distribution of alpha-1-antitrypsin phenotypes in Sweden. *Hum Hered* 1988; **38:** 27–30.
26. Beckman G, Beckman L, Nordenson I. Alpha-1-antitrypsin phenotypes in Northern Sweden. *Hum Hered* 1980; **30:** 129–135.
27. Beckman G, Beckman L, Bjelle A, Dahlqvist SR. Alpha-1-antitrypsin types and rheumatoid arthritis. *Clin Genet* 1984; **25:** 496–499.
28. Fagerhol MK, Eriksson AW, Monn E. Serum Pi types in some Lappish and Finnish populations. *Hum Hered* 1969; **19:** 360–364.
29. Frants RR, Eriksson AW. Reliable classification of six Pi M subtypes by separator isoelectric focusing. *Hum Hered* 1978; **28:** 201–209.
30. Arnaud P, Koistinen JM, Wilson GB, Fudenberg HH. Alpha-1-antitrypsin (Pi) phenotypes in a Finnish population. *Scand J Clin Lab Invest* 1977; **37:** 339–343.
31. Rantala H, Finni K, Similä S. Alpha-1-antitrypsin: the PiM subtypes and serum concentrations in Finnish newborns. *Hum Hered* 1982; **32:** 228–232.
32. Thymann M. Distribution of alpha-1-antitrypsin (Pi) phenotypes in Denmark determined by separator isoelectric focusing in agarose gel. *Hum Hered* 1986; **36:** 19–23.
33. Kellermann G, Walter H. Investigations on the population genetics of the alpha-1-antitrypsin polymorphism. *Humangenetik* 1970; **10:** 145–150.
34. Hoffmann JJ, van den Broek WG. Distribution of alpha-1-antitrypsin phenotypes in two Dutch population groups. *Hum Genet* 1976; **32:** 43–48.
35. Klasen EC, Franken C, Volkers WS, Bernini LF. Population genetics of alpha-1-antitrypsin in the Netherlands. Description of a new electrophoretic variant. *Hum Genet* 1977; **37:** 303–313.
36. Klasen EC, Bos A, Simmelink HD. PI (alpha-1-antitrypsin) sub-types: frequency of PI*M4 in several populations. *Hum Genet* 1982; **62:** 139–141.
37. Blundell G, Frazer A. Alpha-1-antitrypsin phenotypes in Northern Ireland. *Ann Hum Genet* 1975; **38:** 289–294.
38. Arnaud P, Galbraith RM, Faulk WP, Black C. Pi phenotypes of alpha-1-antitrypsin in Southern England: identification of M subtypes and implications for genetic studies. *Clin Genet* 1979; **15:** 406–410.
39. Phillips C, Whitehouse D. A rapid high resolution separator IEF technique for phenotyping alpha-1-antitrypsin. *Adv Foren Haemogenet* 1990; **3:** 274–276.
40. Cook PJL. The genetics of alpha-1-antitrypsin: a family study in England and Scotland. *Ann Hum Genet* 1975; **38:** 275–287.
41. Papiha SS, Pal B, Walker D, Mangion P, Hossain MA. Alpha-1-antitrypsin (PI) phenotypes in two rheumatic diseases: a reappraisal of the association of PI subtypes in rheumatoid arthritis. *Ann Rheum Dis* 1989; **48:** 48–52.
42. Morin T, Martin JP, Feldmann G, Rueff B, Benhamou JP, Ropartz C. Heterozygous alpha-1-antitrypsin deficiency and cirrhosis in adults, a fortuitous association. *Lancet* 1975; **i:** 250–251.
43. Charlionet R, Sesboue R, Morcamp C, Lefebvre F, Martin JP. Genetic variations of serum alpha-1-antitrypsin (Pi types) in Normans. Common Pi M subtypes and new phenotypes. *Hum Hered* 1981; **31:** 104–109.
44. Sesboue R, Charlionet R, Vercaigne D, Guimbretiere J, Martin JP. Genetic variants of serum alpha-1-antitrypsin (Pi types) in Bretons. *Hum Hered* 1978; **28:** 280–284.
45. Youinou P, Gueguen A, Martin JP, Jouquan J, Salmon D, Ruffie J, Le Menn G, Salmon C. Genetic variants of

serum alpha-1-antitrypsin (Pi types) in a Breton population, the Bigoudens. *Hum Hered* 1984; **34:** 114–119.
46. Arnaud P, Chapuis-Cellier C, Vittoz P, Creyssel R. Alpha-1-antitrypsin phenotypes in Lyon, France. *Hum Genet* 1977; **39:** 63–68.
47. Sesboue R, Martin JP. Alpha-1-antitrypsin (PI) polymorphism in France, with special regard to the PI*Z allele. *Hum Hered* 1991; **41:** 340–346.
48. Kimpen J, Legius E, Bosmans E, Van Vaerenbergh V, Ramon A, Raus J. Alpha-1-proteinase inhibitor gene frequencies in Belgium. *Gene Georg* 1990; **4:** 159–163.
49. Fagerhol MK, Tenfjord OW. Serum Pi types in some European, American, Asian and African populations. *Acta Pathol Microbiol Scand* 1968; **72:** 601–608.
50. Klasen EC, Polanco I, Biemond I, Vazquez C, Pena AS. Alpha-1-antitrypsin and coeliac disease in Spain. *Gut* 1980; **21:** 948–950.
51. Goedde HW, Hirth L, Benkmann HG, Pellicer A, Pellicer T, Stahn M, Singh S. Population genetic studies of serum protein polymorphisms in four Spanish populations. *Hum Hered* 1973; **23:** 135–146.
52. Carracedo A, Concheiro L. Distribution of the Pi, TfC, and Gc subtypes in Galicia (North West Spain). *Z Rechtsmed* 1983; **90:** 153–158.
53. Estefania FJ, Carracedo AM, de Pancorbo M, Aguirre AI, Concheiro L. Alpha-1-antitrypsin (Pi) subtypes in the Spanish Basque provinces. *Hum Hered* 1987; **37:** 233–236.
54. Garcia-Orad A, Arizti P, Esteban JL, Garcia-Orad C, Garcia-Arenzana C, Constans J, de Pancorbo MM. Polymorphism of haptoglobin (HP), group specific component (GC) and alpha-1-antitrypsin (PI) in the resident population of the Basque Country (Spain). *Gene Georg* 1990; **4:** 43–51.
55. Martin JP, Sesboue R, Charlionet R, Ropartz C, Pereira MT. Genetic variants of serum alpha-1-antitrypsin (Pi types) in Portuguese. *Hum Hered* 1976; **26:** 310–314.
56. Geada HM, Albino JA, Manso C. Polymorphism of alpha-1-antitrypsin in a Portuguese population. *Hum Genet* 1976; **32:** 109–113.
57. Santos-Rosa MA, Robalo-Cordeiro AJ. Alpha-1-proteinase inhibitor phenotypes in Portuguese population. *Eur J Respir Dis* 1986; **69** (Suppl. 146): 167–173.
58. Goedde HW, Benkmann HG, Christ I, Singh S, Hirth L. Gene frequencies of red cell adenosine deaminase, adenylate kinase, phosphoglucomutase, acid phosphatase and serum alpha-1-antitrypsin (Pi) in a German population. *Humangenetik* 1970; **10:** 235–243.
59. Cleve H, Patutschnick W, Strecker K, Nevo S. Inheritance of PiM subtypes. A study of 151 families with a total of 242 children and of 142 mother–child pairs. *Hum Hered* 1979; **29:** 351–354.
60. Genz T, Martin JP, Cleve H. Classification of alpha-1-antitrypsin (Pi) phenotypes by isoelectrofocusing. Distinction of six subtypes of the PiM phenotype. *Hum Genet* 1977; **38:** 325–332.
61. Bencze K, Sabatke L, Fruhmann G. Alpha-1-antitrypsin: the PiMM subtypes. Do they play a role in development of chronic obstructive pulmonary diseases? *Chest* 1980; **77:** 761–763.
62. Künhl P, Spielmann W. Investigations on the Pi polymorphism in a German population — evidence for two new rare alleles, PiT and Pi<L. *Forens Sci Int* 1979; **14:** 135.
63. Weidinger S, Schwartzfischer F, Cleve H. Pi sub-typing by isoelectric focusing: further genetic studies and application to paternity examinations. *Z Rechtsmed* 1980; **86:** 1–7.
64. Weidinger S, Cleve H, Patutschnick W. Alpha-1-antitrypsin: evidence for a fourth PiM allele. Distribution of the PiM subtypes in Southern Germany. *Z Rechtsmed* 1982; **88:** 203–211.
65. Böhme A, Cleve H, Schönitzer D, Reissigl H, Kazda S, Müller W. Alpha-1-Antitrypsin (Pi) types and subtypes in the Tyrolean population. *Hum Genet* 1983; **63:** 193–194.
66. Bär W, Kratzer A. Polymorphism of alpha-1-antitrypsin (Pi) in the Swiss population determined by isoelectric focusing with an immobilized pH gradient. *Hum Hered* 1988; **38:** 106–110.
67. Pittschieler K, Massi G. Alpha-1-antitrypsin deficiency in two population groups in north Italy. *Padiatr Padol* 1988; **23:** 307–311.
68. Massi G, Cotumaccio R, Auconi P. Alpha-1-antitrypsin (alpha-1-AT) phenotypes and PiM subtypes in Italy. Evidence of considerable geographic variability. *Hum Genet* 1982; **61:** 76–77.
69. Klasen EC, D'Andrea F, Bernini LF. Phenotype and gene distribution of alpha-1-antitrypsin in a North Italian population. *Hum Hered* 1978; **28:** 474–478.
70. Massi G, Fabiano A, Ragusa D, Auconi P, Biagini R, Farinato MA, Bandelloni R. Alpha-1-antitrypsin phenotypes and Pi M subtypes in Italy. *Bull Eur Physiopathol Respir* 1980; **16** (Suppl.): 301–306.
71. Piantelli M, Auconi P, Musiani P. Alpha-1-antitrypsin phenotypes in newborns from Central and Southern Italy. *Hum Hered* 1978; **28:** 468–473.
72. Pascali VL, De Mercurio D. Determination of alpha-1-antitrypsin subtypes in the population of Rome: a study in ultrathin-layer isoelectric focusing. *Hum Hered* 1981; **31:** 296–298.
73. Klasen EC. Alpha-1-antitrypsin phenotype and gene distribution in Southern Italy. *Hum Hered* 1981; **31:** 180–182.
74. Davrinche C, Charlionet R, Rivat C, Sesboue R, Bayorek M, Arnone M. Genetic variants of human B component (BF system) and alpha-1-antitrypsin (PI system) in a population from Sardinia. *Hum Hered* 1984; **34:** 297–302.
75. Jelić-Ivanović Z, Spasojević-Kalimanovska V, Topić A, Spasić S, Petrović V. Alpha-1-antitrypsin (Pi) polymorphism in Serbia: deviation of Pi M subtype distribution from the Hardy–Weinberg equilibrium. *Gene Georg* 1994; **8:** 129–135.
76. Fertakis A, Tsourapas A, Douratsos D, Angelopoulos B. Pi phenotypes in Greeks. *Hum Hered* 1974; **24:** 313–316.

77. Petridou E, Chapuis-Cellier C, Roukas K, Lan S-J, Tzonou A, Trichopoulos D. Tobacco smoking and other factors in relation to serum alpha-1-antitrypsin. *Hum Biol* 1993; **65:** 425–432.
78. Benkmann HG, Timar L, Szentesi I, Wimmer U, Lohmann E, Czeizel A, Goedde HW. Ecogenetic and pharmacogenetic studies in Hungary. *Gene Georg* 1993; **7:** 203–212.
79. Szczeklik A, Turowska B, Czerniawska-Mysik G, Opolska B, Nizankowska E. Serum alpha-1-antitrypsin in bronchial asthma. *Am Rev Respir Dis* 1974; **109:** 487–490.
80. Kowalska A, Rujner J. Polymorphism locus Pi (alpha-1-antitrypsin) of residents in the Poznan province. *Pol Tyg Lek* 1994; **49:** 195–197.
81. Shurkhal AV, Podogas AV, Altukhov YP. Genetic polymorphism and rare variants of alpha-1-antitrypsin in the population of Moscow. Research using isoelectric focusing on an ultrathin gel. *Genetika* 1984; **20:** 2066–2069.
82. Antonov MP, Petrukhin IS, Voitova TV, Portenko GM. Distribution of Pi phenotypes in normal subjects and subjects with diseases of the respiratory tract. *Lab Delo* 1988; **3** (10): 23–27.
83. Hubbard RC, Sellers S, Czerski D, Stephens L, Crystal RG. Biochemical efficacy and safety of monthly augmentation therapy for alpha-1-antitrypsin deficiency. *J Am Med Assoc* 1988; **260:** 1259–1264.
84. Sofaer JA. Population genetics (Hardy–Weinberg equilibrium and factors affecting it). In: Emery AEH, Rimoin DL, eds. *Principles and Practice of Medical Genetics*, 2nd edition. Edinburgh: Churchill Livingstone, 1990, chap. 9.
85. Hutchison DCS, Tobin MJ, Cook PJL. Alpha-1-antitrypsin deficiency: clinical and physiological features in heterozygotes of Pi type SZ. A survey by the British Thoracic Association. *Br J Dis Chest* 1983; **77:** 28–34.
86. Diem K (ed.). *Documenta Geigy Scientific Tables*, 6th edition. Manchester: Geigy Pharmaceutical Co. Ltd.
87. DeCroo S, Kamboh MI, Ferrell RE. Population genetics of alpha-1-antitrypsin polymorphism in US whites, US blacks and African blacks. *Hum Hered* 1991; **41:** 215–221.
88. Byth BC, Billingsley GD, Cox DW. Physical and genetic mapping of the serpin gene cluster at 14q32.1: allelic association and a unique haplotype associated with alpha-1-antitrypsin deficiency. *Am J Hum Genet* 1994; **55:** 126–133.
89. Mourant AE, Kopeć AC, Domaniewska-Sobczak K. *The Distribution of the Human Blood Groups and Other Polymorphisms*, 2nd edition. London: Oxford University Press, 1976.
90. Cavalli-Sforza LL, Menozzi P, Piazza A. *The History and Geography of Human Genes*. Princeton, NJ: Princeton University Press, 1994, chap. 5.
91. Sveger T. Liver disease in $alpha_1$-antitrypsin deficiency detected by screening of 200 000 infants. *N Engl J Med* 1976; **294:** 1316–1321.
92. Piazzi A, Cappello N, Olivetti E, Rendine S. A genetic history of Italy. *Ann Hum Genet* 1988; **52:** 203–213.