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Review Synthetic Report

Origins and spreads of Alpha 1 antitrypsin variants in world human populations: a synthetic review

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Abstract - Human Alpha 1 antitrypsin (AAT) or protease inhibitor (PI) is a highly polymorphic glycoprotein. In this review report distributions of all detected AAT allelic variants in different world populations are collected showing their anthropological usefulness. Some variants are related to AAT deficiency (AATD) which is one of the most common genetic disorders worldwide. Data on PIS and PIZ, most common AATD mutations, and their worldwide distribution are also reported. Studies on DNA haplotypes were particularly useful for the estimations of PIS and PIZ mutations ages and worldwide spread. According to these data, it seems very likely that the PIZ mutation occurred in Sweden and was spread into the rest of the European continent via the Baltic countries. However, in a second hypothesis authors suggested multiple origins of this mutation. The highest frequencies of the PIS mutation in the Iberian Peninsula were linked to an early occurrence of the mutation in this region, before its spread throughout the Europeans countries and the Iberian Peninsula settlements and trading networks. However, the PIS allele may have been further dispersed by multiple occurrences of the mutation. This multiple origins of AAT variants could explain the higher frequencies and haplotype diversities in PIM1Val and PIM2 alleles, rather than their older base alleles (PIM1Ala and PIM3, respectively). Data on rare variants, which seem not to be so rare in some populations, are still lacking. The only reliable data, on the age of a rare AATD variant, was given on an autochthonous allele (NullOurém).

Key words: Alpha 1 antitrypsin, allelic variants, worldwide distribution, origins and spread of mutations.

Introduction

The alpha1-antitrypsin (AAT), the most important general protease inhibitor (PI), is a highly polymorphic glycoprotein with more than 120 allelic variants belonging to a genetic system firstly designated "system PI". These variants are generally classified alphabetically by the PI nomenclature, in terms of their electrophoretic mobility (Fagerhol and Laurell, 1970): for example the PIB variant is the most anodal, PIM the middle and most common one, and PIZ the most cathodal.

Some of these allelic variants are associated with deficiency in AAT. This alpha 1 antitrypsin deficiency (AATD) represents a common hereditary disorder that mainly manifests as obstructive lung diseases and less common liver diseases. The AAT is a 52 kDa molecule synthesized mainly by hepatocytes. This is the main serine-protease inhibitor in the plasma, with normal circulating concentrations showing intra and inter-populations variation in a range from 0.9 to 3.5 g/L. The AAT is an acute phase protein, characterized by a rapid increase in its synthesis during the acute phase of inflammation, to address the imbalance of the protease-antiprotease balance, caused by increased proteolytic activity at the inflammation site (Jeppsson and Franzen, 1982). Despite the dissemination of AAT in all organs of the body, its main function is to be exercised in lung parenchyma, where it serves to protect the fragile alveolar tissue destruction by neutrophil elastase (NE), a powerful protease that destroy the major structural proteins of the cells.

Mutations in the PI gene (also called *SERPINA1*), which encodes the AAT, are the cause of abnormal synthesis and secretion of AAT. Several AAT mutations are simple polymorphisms that not affect neither the amount of protein in serum nor its function (M variants: M1 (Ala213Val GCG→GTG), M2 (Arg101His CGT→CAT) and M3 (Glu376Asp GAA→GAC) are the most common normal variants). Some variants (I (Arg39Cys CGC→TGC), S (Glu264Val GAA→GTA), P (Val256Asp GAT→GTT)) are accompanied by a slight decrease in protein concentration (60 to 70% of normal rate). Other variants (Z (Glu342Lys GAG→AAG), Mmalton (ΔPhe52 delTTC) are accompanied by a dramatic decrease of AAT (less than 20% of normal rate) or its total disappearance (the null variants as Nullbellingham (Lys217AAG →StopTAG) (Crystal et al., 1990). PIS and PIZ AATD alleles are the most commonly encountered while other variants are rarely reported (Luisetti and Seersholm, 2004).

Alpha 1 antitrypsin gene evolution

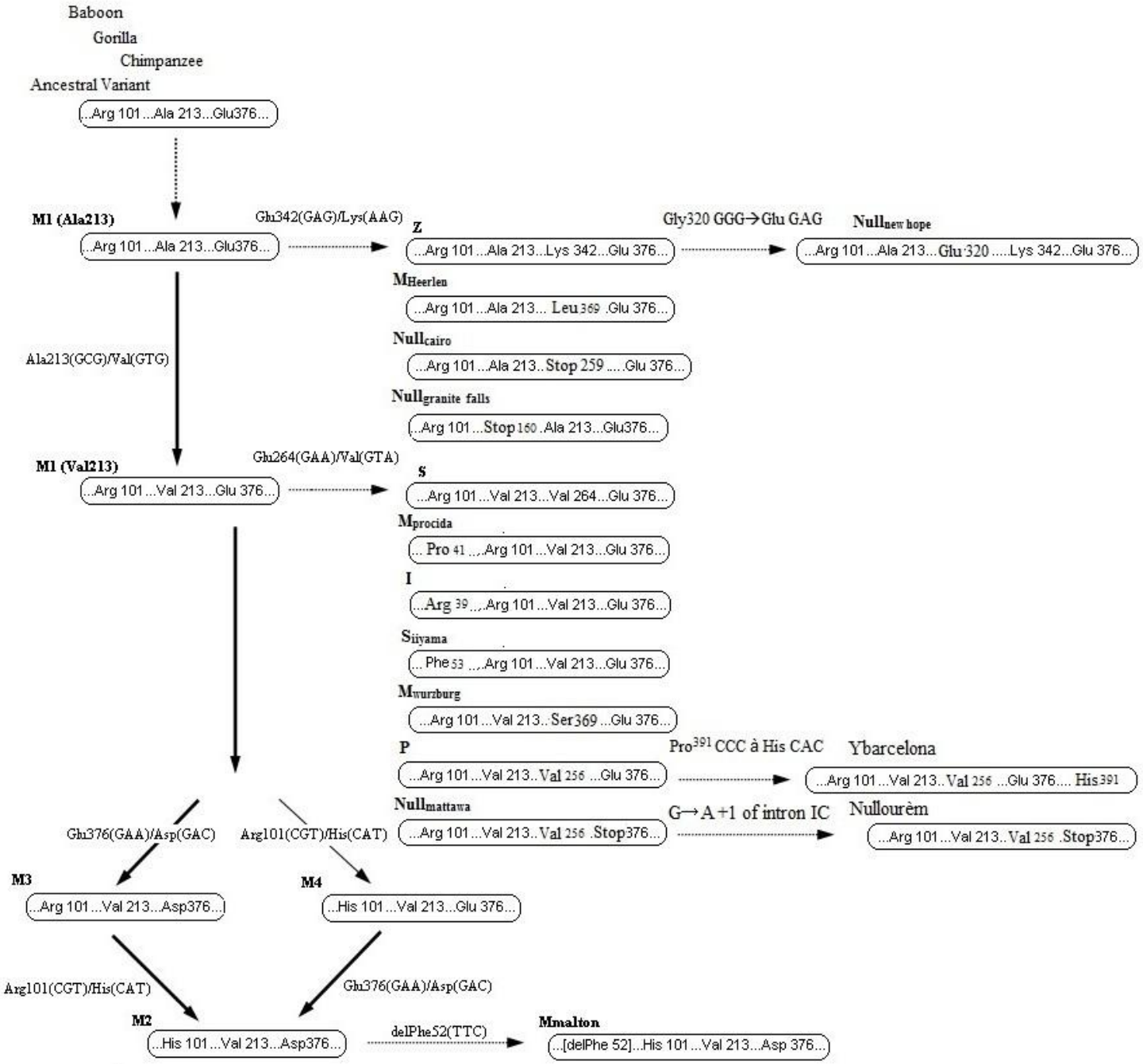
The serpins (Serine Protease Inhibitors) family regroups more than 700 constituents. Several data, related to the serpin genes evolution and variations in mammals between humans and higher primates, exist. The PI gene is located on chromosome 14q32.1, within the serine protease inhibitor cluster. This region, spanning approximately 320 kb, also includes α1-antitrypsin pseudogene sequence (PIL), α1-antichymotrypsin (ACT), corticosteroidbinding globulin (CBG), protein C inhibitor (PCI) and kallistatin (PI4) Genes.

The similarities of the exon-intron structures between the AAT and alpha 1 antichymotrypsin and their proximity to the 14q31 position indicates the recent divergence of these genes (about 100 to 250 million years ago) (Bao et al., 1987).

The sequencing of coding exons (exons II to V) of the AAT in the chimpanzee and the gorilla has shown that there is one difference in one amino acid from human AAT M1 (Ala213) (a difference between the protein in position 385: Methionine in human, Valine in the chimpanzee) (Nukiwa et al., 1996).

Since the base alleles of most mutation were clearly established and according to parsimony principle, it becomes possible to establish a phylogenetic tree of the AAT variants (Figure1).The PIM1 allele (Ala213) is the initial allele, all other derived by one or more substitutions. Within this tree, the PIM4 allele could have been arisen either on PIM1 (Val213) or on PIM2 alleles or from intragenic recombination between both.

Figure 1. Phylogenetic tree of the AAT variants based on the parsimony principle, modified from Nukiwa et al., 1996.



AAT gene variants and their use in anthropological investigations

Studies using direct DNA analysis are now abundant. In some of these studies nuclear DNA polymorphisms were analyzed in a large number of loci and in others particular genetic materials such as those of maternal (mtDNA) or paternal (Y chromosome). However, in the first genetic investigations of human populations authors have used classical genetic markers such as those of ABO blood groups, the PI system of alpha 1 antitrypsin, HLA Loci, Rhesus system and particularly those of immunoglobulin GM system known by its unparalleled ability, of a single system, to differentiate human populations (for review see Chaabani, 2002).

The frequency of different AAT alleles was widely used to characterize populations. The results obtained from studies, investigating the isoelectrofocusing technique (IEF) to discriminate between AAT variants, showed different allelic distribution among populations. These results, some of which are recapitulated in Table 1, show that sub-Saharan African populations were characterized by a relatively high frequency of PIM1 allele, which is less high in the European populations, and low presence of PIS and PIZ alleles. Furthermore, the molecular analysis of the two IEF-indistinguishable alleles PIM1Ala and PIM1Val showed that the ancestral PIM1Ala variant is more present in sub-Saharan subjects than in Europeans (Gaillard et al, 1994; Hayes, 2003). The Indian populations of South America were characterized by the high presence of PIM3 allele. On the other hand, PIM2, PIS and PIZ alleles were reduced. The European populations presented relatively high frequencies of the PIM2, PIS and PIZ alleles. This high presence of the PIM2 allele was also shared by the North African, Middle Eastern and Asian populations. On the contrary, PIS and PIZ alleles were rarely described in these populations.

It is generally agreed that sub-Saharan Africans show more genetic diversity than Europeans and are therefore believed to be older (Jorde and Wooding, 2004) which confirms the high frequency of the ancestral PIM1Ala allele within the African population. The high presence of the PIM3 allele in the American Indian could be attributed to a genetic drift in this population likely originated from Central Asia immigrants around 40.000-16500 years ago (Spencer W and Mark R, 2002). However, an earlier immigration of these people before the PIM2 mutation occurrence in the other populations cannot be excluded.

Table 1. Allele frequencies of PI system in human populations.

Population	***Frequencies***						***References***
	PIM1	PiM2	PIM3	PIS	PIZ	Other	
Italy	0.653	0.181	0.118	0.028	0.010	0.009	Pascali and DeMercurio, 1981
France	0.6675	0.142	0.100	0.063	0.018	0.006	Constans et al.,1980
Iberian Peninsula	0.538-0.750	0.106-0.204	0.006-0.115	0.064-0.149	0-0.023	-	Moral et al., 1997
Sardinia	0.607	0.221	0.126	0.040	0.006	-	Walter et al., 1989
Sweden	0.694	0.138	0.114	0.024	0.023	0.006	Hjalmarsson, 1988
Denmark	0.728	0.136	0.082	0.022	0.023	0.009	Thymann, 1986
Poland	0.720	0.161	0.096	0.009	0.006	0.006	Kowalska et al., 1995
White Americans	0.640	0.190	0.110	0.042	0.013	0.006	Kuppers and christopherson, 1978
Black Americans	0.903	0.028	0.054	0.005	0.003	0.007	Kuppers and christopherson, 1978
Mali	0.930	0.020	0.040	0	0.010	0	Frants and Ericksson, 1978
Kenya	0.980	0.020	0	0	0	0	Frants and Eriksson, 1976
Tunisia	0.800	0.150	0.040	0.006	0	0	Denden et al., 2008
Libya	0.623	0.205	0.132	0.005	0	0.032	Sebetan, 1992
Morocco	0.730	0.126	0.072	0.058	0	0.014	Harich et al., 2002
Jordan	0.666	0.158	0.150	0	0	0.065	Cleve et al., 1982
Iran	0.729	0.161	0.107	0.003	0	0	Walter et al., 1992
Bolivia	0.592	0	0.391	0	0	0.016	Frants, 1980
Brazil	0.696	0	0.304	0	0	0	Frants, 1980
Venezuela	0.467	0.004	0.529	0	0	0	Marini et al., 1993
China	0.659	0.245	0.087	0	0	0.01	Lee et al., 1981
Korea	0.649	0.219	0.061	0	0.006	0.02	Lee et al., 1981

In our work, investigating AAT variants distribution in a population from central Tunisia (Denden et al., 2008), the frequency of PIM1 allele was 0.80, while those of PIM2 and PIM3 were 0.15 and 0.04, respectively. This consistency of our results with those observed in the European population was further elucidated by the PIM1 allele distribution in our sample: PIM1Val frequency (0.78) was higher than that of PIM1Ala (0.22) (data not published). However, we found PIS and PIZ allelic frequencies (0.006 and 0, respectively) in agreement with the data found in sub-Saharan Africans. These results support the rarity of PIS and PIZ most common deficient alleles in the North African population that was noted in other studies. Makni et al., (1997), in a study on a population from the North of Tunisia, reported the scarcity of PIS allele (allele frequency of 0.01) while the PIZ allele was not detected.

Data from the Libyan population are also in favor of PIS and PIZ allele rarity in the South-Mediterranean area (Sebetan, 1992). However, the high frequency of PIS variant in Morocco lies to the data of North African peoples (Harich et al., 2002). This finding would indicate a close relationship between Moroccan and Iberian Peninsula populations, where this allele is the most prevalent worldwide. In their studies on Berber groups from Tunisia, Chaabani et al., (1984, 1988) found that the frequencies of AAT alleles are ranged from (0.64 to 0.70), (0.09 - 0.27) and (0.07 to 0.19) for PIM1, PIM2 and PIM3 alleles, respectively, in the different groups studied. PIZ has not been identified, while another variant, slightly cathodal to S variant, was detected and firstly designated PISberber. However, it was found to be indistinguishable from PIWsalerno (Cook, 1975) which was originally described in an Italian family from Salerno.

These data show the genetic relationship between populations of the Mediterranean region which reflects at least some people movements between the two sides of Mediterranean during historic periods. In addition, another scarce variant PIPclifton was detected in some Berber groups. This variant, which had been reported in a North American population originated from sub-Saharan Africa (Hug et al., 1981), is often associated with immunoglobulin GM and Cγ haplotypes frequent in sub-Saharan African populations. Thus, it seems to be typical to these populations (Chaabani et al., 1986) and shows the presence of sub-Saharan African traces in the gene pool of North African populations. This suggestion agrees with those deduced from many other classic and molecular markers analyses (e.g., El Moncer et al., 2010).

As show these examples of certain allelic variants that occurred in single population could be served as unique population markers, but their presence in exceptional low frequencies limits their anthropological usefulness.

Worldwide distribution of Alpha 1 antitrypsin deficiency variants

Alpha 1 antitrypsin deficiency (AATD) is a common genetic disease that affects one out of 2000-5000 people (Stoller and Aboussouan, 2005). The population-based studies, on the prevalence of AAT deficiency worldwide, were based on the distribution of the most common PIS and PIZ allele frequencies. Early studies showed that PIS and PIZ alleles frequencies are relatively high (0.01-0.02) in Europeans, whereas these variant are very rare or completely absent in other populations. One explanation for this finding could be the small size of the cohorts analyzed in countries outside of Europe and America, where expert diagnosis laboratories are generally lacking.

However, in some developed countries such as South Korea and Japan, only a few cases of PIS and PIZ alleles related AAT deficiency have been identified in European descendants (Nukiwa et al., 1996). In Japan, the most common deficiency variant is PISiiyama (Ser53Phe), which is present in most AAT deficient patients (Seyamaet al., 1995). The studies of sub-Saharan Africans and Americans having sub-Saharan African origin have also demonstrated the scarcity or total absence of PIS and PIZ allele in these populations (Pierce et al., 1975; Kuppers and christopherson, 1978). Nevertheless, the study of Spinola et al (2010), on a sub-Saharan African population (Cape Verde), showed that the PIS mutation presents one of the highest frequencies (3.2%) in sub-Saharan Africans, but lower than Angolans (18.8%), Namibians (14.7%), Nigerians (6.4%) and Botswains (4.5%). The highest frequency of the PIS allele worldwide is found in southern Europe peaking in the Iberian Peninsula with a mean gene frequency of 0.0564, suggesting the mutation is likely to have arisen in this region (Martin et al., 1976; Carracedo andConcheiro, 1983).

Interestingly, the highest frequencies of PIS alleles in sub-Saharan African population are reported in geographic areas sharing historical relationship with Portuguese (settlement, exploration expeditions or trading networks).

Cape Verde shows a PIZ allele frequency (0.2%) lower than other sub-Saharan African populations, namely Somalia (1.15%), Mali (0.98%) or Nigeria (0.36%). However, many other sub-SaharanAfrican populations, like Botswana, Congo, Cameroon, Angola, Gambia, Mozambique and Namibia, lack the PIZ mutation.

The meta-analysis performed by de Serres et al. (2007), based on data collected from different cohorts studied for AAT deficiency in the world, have shown that PIS and PIZ alleles-related AATD not only affects individuals of Northern Europe but the sub-Saharan Africans, Arabs, Jews of Middle Eastern and Asian populations. However, the prevalence of the disease remains highest in European and their descendants in Australia, New Zealand, South Africa and North America. The study of Blanco et al. (2006), performed in the European area, showed that the largest numbers of PIZZ individuals were found in Italy, Spain, Germany, France, the UK, Latvia, Sweden and Denmark, with 5,000–15,000 individuals in each of these eight countries. On the contrary, the lowest number of individuals with the PIZZ phenotype was found in Finland, Hungary, Poland, Estonia, Lithuania and Switzerland (<1,000 for every of these six countries).

The highest prevalence of both PIS and PIZ alleles in the same population were shown in an isolate from Portugal (Madeira Island). PIS mutation has 18 %, and PIZ mutation (2.5%) was the third highest worldwide. The frequency of AAT deficiency genotypes in Madeira (PIZZ, PISS, and PISZ) is estimated to be the highest in the world: 41 per 1000 (Spinola et al., 2009). A founder effect, in this isolated area, would explain this high prevalence of the PIS and PIZ mutations.

AAT deficiency remains an underdiagnosed disease worldwide, and many areas are still unexplored. Meta-analysis of existing data indicates a higher prevalence of PIS and PIZ alleles in populations of northern Mediterranean populations over North Africa and the Middle East. The interpretation of these results is still limited by the large difference in the number and size of the analyzed cohorts in both sides of the Mediterranean Sea. In addition, it seems very likely that targeted screening programs (screening of subjects at risk) have a higher detection rate of AAT deficiency compared with population-based studies (Stoller and Aboussouan, 2009). In a study from Ireland (Carroll et al., 2011), they found that the frequency of the PIS allele in a random sample from the general population was similar to that identified in the targeted population.

However, the frequency of the PIZ allele was four-fold higher in the targeted populationcompared to the general population.

Moreover, rare AAT variants remain an unexplored condition worldwide. Screening for AATD performed in 120 Tunisian patients with obstructive lung diseases (Denden et al., 2009) noted a rare allele frequency (5/120) larger than that reported in the targeted screening program in Italy (37/2922) (Ferrarotti et al., 2005) while PIZ allele was detected only once. This finding of relatively high frequency of rare AATD variants was established in the Central and Southern Italy, where the frequency of Mmalton and Mprocida alleles is higher than that of common PIS and PIZ alleles (Ferrarotti al, 2005). The Mmalton allele, which represents 3/5 cases of rare variants identified in patients from Tunisia, is the most common variant in Sardinia where the PIZ allele is very rarely encountered (Orrù et al., 2005). A new variant nullcairo (Zorzetto et al., 2005), described for the first time in an Egyptian family, was detected among unrelated individuals in southern Italy. The population movements such as emigration, invasions or contacts for trade during different historic periods could favor the spread of these variants in the Mediterranean area.

These data suggest that rare AATD variants may be more prevalent in areas where the common deficiency PIS and PIZ variants are less common (Stolk et al., 2006). However, In Switzerland, where PIS and PIZ AATD alleles are common, a high prevalence of rare AAT alleles (2.8%) has been reported, with PIMwurtzburg allele (Pro369Ser) as the most common (13% of rare alleles) (Zorzetto et al., 2008). In a recent study concerning the Spanish population (Rodriguez-Frias et al., 2012), where the largest number of PIZZ individuals worldwide was found, 77 cases with rare AATD alleles over 3511 AATD cases were reported. In this study, there was a higher presence of two deficient alleles, PII (Arg39Ser) and PIMmalton, found in 26 (34%) and 15 cases (20%), respectively. As reported by the authors, the results found in Spain were similar to those reported in Ireland by Carroll et al., (2011), where PII variants accounted for 90% of the total rare AATD variants. This observation, together with the similar frequency of PIS (0.05) in Spain and Ireland, suggests ethnic relationships between the two countries.

Even some AATD rare variants seems to be autochthonous to specific areas (PIbarcelona and Mvalld'hebron in Spain, PISiiayama in Japan), the data are in agreement with AATD rare allele interchange between populations. However, more

future studieswill be necessary to elucidate the mutations origins (unicentric or multicentricorigin) and worldwide spread.

Ages and origins of Alpha 1 antitrypsin allelic variants

Ages and origins of AAT variants were investigated in several studies on European populations (Table 2), where PIS and PIZ AATD alleles are the most prevalent worldwide. PIZ mutation haplotype analysis was firstly conducted by Cox et al (1985) on British, Ukrainian, German, Dutch and French patients carrying the mutation. The authors showed that the PIZ allele occurs with a specific unique RFLP haplotype and indicated a single relatively recent origin of the mutation in European, which was predicted to be an individual who lived in Northern Europe about 6840 years.

Table 2.
Ages and origins estimates of AAT variants in different European populations

AAT Variant	*Population*	*Age (years)*	*Origin*	*Haplotype analyzed*	*References*
PIZ	British, Ukrainian, German, Dutch and French 0.653	6840	Northern Europe	RFLP	Cox et al., 1985
PIZ	Northern European	2000	Northern Europe	RFLP, STR	Byth et al., 1994
PIZ	Swedish	14000	Southern Sweden	-	Hutchison, 1998
PIZ	Portuguese	4070	Non scandinavian origin	STR	Seixas et al., 2001
PIZ	Latvian	2902	Sweden	RFLP,SNP	Lace et al., 2008
PIZ	Swedish	2362	Sweden	RFLP,SNP	Lace et al., 2008
PIS	Portuguese	14100	Iberian Peninsula	STR	Seixas et al., 2001
PIS	Basque	8370	Iberian Peninsula	STR	Seixas et al., 2001
PINullOurém	Portuguese	650	Portugal	STR	Vas Rodriguez et al., 2011
PIM3	Portuguese	22170	-	STR	Seixas et al., 2001
PIM3	Basque	18750	-	STR	Seixas et al., 2001
PIM2	Portuguese	16080	-	STR	Seixas et al., 2001
PIM2	Basque	13590	-	STR	Seixas et al., 2001

Using 12 polymorphic restriction sites with seven different restriction enzymes, the analysis, reconducted with rare AATD variants (PIMmalton, PIMcobalt, PIMduarte, Nullmattawa, Nullludwigshafen, Nullhongkong and Nullbellingham) showed different DNA haplotypes for each of types. All of the rare deficiency alleles can be distinguished from PIZ by their DNA haplotype, and most can be distinguished from each other (Cox and Billingsley, 1989). In 1994, Byth et al. recalculated the age of the PIZ allele to be 2000 years or 66 generations, by analyzing STR and RFLP markers on the serine protease inhibitor cluster 14q32.1 in Northern European PIZ carrying subjects. Hutchison (1998) linked the higher prevalence of PIZ allele in Sweden, to a founder mutation arisen in the southern part of Sweden, probably about 14,000 years ago. Beckman et al. (1999) reported that the western part of Latvia (Courland) showed the highest frequency of the PIZ allele identified to that date and explained his finding by archaeological data on the settlement in this part of people from Sweden after the seventh century.

In another study on two Northern European populations (heterozygous and homozygous PIZ allele carriers from Latvia (n = 21) and Sweden (n = 65), and unrelated healthy donors from Latvia (n=113)), Lace et al. (2008) analyzed 3 SNP and 2 *SacI* restriction enzyme sites in the *SERPINA1* gene. Similar frequencies and patterns were observed in PIZZ homozygous individuals from Latvia and Sweden indicating common ancestry. The ages of the PIZ allele were estimated to 2902 years (97 generations) and 2362 years (79 generations) in Latvia and Sweden, respectively. According to these age estimates in different European regions, it seems very likely that the PIZ mutation occurred in Sweden and was spread into the rest of the European continent via the Baltic countries.

The analysis of four STR markers along the 14q32.1 PI cluster in populations from Portugal with different historical backgrounds (Portuguese and Basque) showed different PIZ mutation age estimation derived from each of the STR markers (Seixas et al., 2001). Although the value derived from corticosteroid-binding globulin STR tend to be lower than that calculated from AAT STR, age estimates for PIZ based on these loci (4070 years) lies between previous estimates in Scandinavian (Byth et al., 1994) and European population (Cox et al., 1985). However, the observation of similar or even higher STR diversity in PIZ types from Portugal compared to those found in Northern

Europe contrasts with a unique Northern Europe origin of the mutation in Portugal and may indicate its multiple occurrences in Europe.

The multiple origin of PIZ mutation is strengthened by the study of Chappell et al. (2004), investigating 16 *SERPINA1* SNPs in 41 PIZZ genotype carrying individuals from the UK, in which they found that the major haplotype in PIZZ individuals (81%) carries the PIM1Ala213 allele, as originally described (Nukiwa et al., 1986) while 4 of the less common PIZZ haplotypes (6%) can be seen to carry the PIM1Val213 allele. This finding is also shared by the Swiss population, where the mutation was linked to the PIM1Val213 allele in 2% of cases (Zorzetto et al., 2008). However, the hypothesis of another occurrence of the C to T (Ala213Val) mutation on an ancestral haplotype originally carrying the PIZ mutation cannot be excluded.

On the basis of the observation of the highest PIS frequencies in the Iberian Peninsula, it has been considered the most likely place of mutation origin. Seixas et al. (2001) estimated the mutation age in Portugal (14,100 years) and in Basque countries (8370 years). This finding indicates that the high frequencies of this variant in the Iberian Peninsula is due to an early occurrence of the mutation in this region and that it could have predated the pronounced differentiation of the Basque population. However, in their study on the Swiss population, Zorzetto et al. (2008) reported that 10% of the PIS mutations were seen to carry the ancestral PIM1 (Ala213) allele, rather than the originally described base allele (PIM1 Va213) (Curiel et al., 1989). The PIS allele may have been further dispersed by multiple occurrences of the mutation.

The only reliable data, on a rare AATD variant age, was given by Vas Rodriguez et al (2011) on an autochthonous allele (NullOurém). To characterize NullOurém background haplotype, the authors analyzed seven microsatellites from *SERPINA1* gene. The mutation was estimated to have arisen 650 years ago, in the 14th century (1360). Rare variants seem not to be so rare in some populations. More data are still needed to elucidate their dispersion in specific area.

Tracing the evolutionary history of normal AAT variants is of great interest to explain the different allelic distribution in human populations. Seixas et al. (2001) studied normal AAT variants (PIM1Ala, PIM1Val, PIM2 and PIM3) haplotypes in two populations from the Iberian Peninsula. Age estimates of PIM3 allele in the Portugal and Basque samples were of 22170 and 18750 years, respectively. The occurrence of PIM2 mutation in these populations was estimated around 16080 and 13590 years ago, respectively. According to the authors, these variants had lower age estimates than would be expected from their wide geographical distributions, suggesting that their dispersion in Europe might have been preceded by important bottlenecks upon the African Ancestor or may indicate a different origin of the PIM3 allele in Europe. The multiple origins of AAT variants could explain the higher frequencies and haplotype diversities in PIM1Val and PIM2 alleles, rather than their older base alleles (PIM1Ala and PIM3, respectively). More extensive data on these variants, involving sub-Saharan African, European, Asian and American Indian populations, will be needed to provide reliable data on their spread.

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