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Protease Inhibitor Frequencies: Is There a Deficit of M Subtype Heterozygotes?

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Abstract. A large number of reports on human protease inhibitor (PI) type frequencies in various populations have now appeared. A combination of the results of published studies shows that the observed frequencies of the M subtype homozygotes and heterozygotes differ significantly from those predicted by the Hardy-Weinberg equilibrium, especially among Europeans but probably not among Asians or Africans. The M1, M2 and M3 homozygotes are more numerous than would be expected, while the M1M2 and M1M3 heterozygotes are less common than expected.

The plasma protein α_1-antitrypsin, which inhibits the activity of elastase and other proteases, exists in a number of genetically determined forms. Some of the protease inhibitor (PI) types, especially Z and S, are associated with decreased circulating levels of the protein and this can lead to disease in some circumstances [1, 2]. However, variation within the M type does not seem to be associated with deficiency although there may be differences in mean inhibitor concentration and activity between the various homozygotes and heterozygotes [3, 4]. Some disease associations of particular M subtypes have been reported [5, 6] and a recent study found that subjects who had survived to old age were less likely to be heterozygous for M subtype than would be expected from the frequency in younger subjects [7].

Most reports on PI M subtype frequencies in healthy populations state that frequencies of homozygotes and heterozygotes are compatible with those expected under the Hardy-Weinberg equilibrium, but a very large number of subjects would be needed to avoid beta error – the erroneous conclusion of no difference when one does in fact exist. A number of studies have recorded slightly more M homozygotes, and slightly fewer M heterozygotes, than expected. The possibility that this is a true deviation from the expected equilibrium has been tested by combining results from individual published reports.

Table 1. Overall observed and predicted frequencies of PI M homozygotes and heterozygotes in 24,303 subjects reported in earlier studies [4, 5, 8–43]

Group	M1	M2	M3	M1M2	M1M3	M2M3
European						
Observed	9,693	507	210	3,354	1,650	397
Expected	9,443	367	126	3,657	1,843	380
$(O-E)^2/E$	6.62	53.41	56.00	25.11	20.21	0.76
African						
Observed	1,011	10	2	60	82	6
Expected	1,003	2	3	76	82	5
$(O-E)^2/E$	0.06	32.00	0.33	3.37	0	0.20
Asian						
Observed	2,132	197	21	1,260	332	99
Expected	2,138	201	16	1,257	342	97
$(O-E)^2/E$	0.02	0.08	1.56	0.01	0.29	0.04

The subjects are grouped by area of geographic and/or genetic origin.

Methods

Reports [4, 5, 8–43] were selected from the literature which fulfilled the following criteria: distinction between at least M1, M2 and M3 homozygotes and the M1M2, M1M3 and M2M3 heterozygotes (few quantitative reports on M4 have appeared); reports of the absolute numbers of subjects in each group, and a sample not selected for any illness. Most reports also gave information about the frequency of the S and Z alleles and the number of subjects who were M1S, M1Z, M2S etc.

This yielded 24,303 individuals whose PI type had been determined. Most groups studied were blood donors or persons involved in paternity disputes, and therefore likely to be young adults. Where the expected and observed numbers in each group were given in the paper these figures were used; where only the observed numbers were given the expected numbers have been calculated from the gene frequency in that sample. The numbers of subjects of each identified PI type observed and expected were summed across the various reports, first globally and then by major geographic and racial groups.

Results

Taking all reports together, there was a highly significant deviation from the expected frequencies ($\chi^2 = 204.0$, 11 d.f., $p < 0.001$). In tables 1 and 2, results are grouped by geographic area; where populations are largely nonindigenous (e.g. USA, Australia) they are grouped with others of similar genetic background. The numbers of subjects observed and expected with each phenotype are summed for the European, African and Asian

Table 2. Overall observed and predicted frequencies of PI MS and MZ types in 24,303 subjects reported in earlier studies [4, 5, 8–43]

Group	M1S	M2S	M3S	M1Z	M2Z	M3Z
European						
Observed	797	163	96	338	70	44
Expected	803	160	91	345	66	34
$(O-E)^2/E$	0.04	0.06	0.27	0.14	0.24	2.94
African						
Observed	7	0	0	2	0	0
Expected	7	0	0	2	0	0
$(O-E)^2/E$	0	0	0	0	0	0
Asian						
Observed	0	0	0	0	0	0
Expected	0	0	0	0	0	0
$(O-E)^2/E$	0	0	0	0	0	0

The subjects are grouped by area of geographic and/or genetic origin.

groups and the χ^2 statistic calculated for each.

In the European group, there was a highly significant divergence from the expected pattern of types ($\chi^2 = 165.8$, 11 d.f., $p < 0.001$). Of the M homozygotes, there were 2.7% more M1 than expected, 39.2% more M2 and 70.2% more M3. There were corresponding reductions in the numbers of M1M2 and M1M3 heterozygotes.

Of 30 reports on populations of European descent, 24 showed more M1 homozygotes than predicted from the gene frequencies, 5 showed fewer and 1 the same. This number of positive (i.e. observed > predicted) results is significantly greater than can be explained by chance ($p < 0.01$, sign test).

Among Asian populations, however, this anomaly did not occur. It is not possible to reach a conclusion about the African group because of the much lower frequencies of the M2 and M3 genes; although the value of χ^2 is high because of an excess of M2 homozygotes, the expected frequency in this cell is only 2 and the χ^2 test is therefore unreliable.

Discussion

This summary of published reports on PI M subtype frequencies reveals that there is a significant deviation from the expected proportions of the different types. Individual reports do not show a significant effect because they each considered smaller numbers of subjects. The most notable deviations from the expected numbers are an excess of M1, M2 and M3 homozygotes and a deficit of M1M2 and M1M3 heterozygotes. The M2M3 heterozygotes are present in slightly more than the expected numbers, as are the M2S, M3S, M2Z and M3Z types. Overall, the pattern is that M1 in combination with any other subtype or type is less frequent than would be expected.

A number of possible explanations need to be considered. Firstly, a null allele could lead to an excess of apparently homozygous phenotypes. Such null alleles do exist but appear to be rare. Beckman and Beckman [3] considered this possibility as an explanation for low concentrations of α_1-antitrypsin in the plasma of some apparently M1, M2 or M3 individuals, but as the concentrations were more

than half the modal value for M homozygotes they rejected it. In addition, a null allele would affect all combinations of M1, M2 and M3 proportionately, whereas in fact (unlike M1M2 and M1M3) M2M3 does not occur less frequently than expected.

Secondly, a widespread technical error in the typing in which heterozygotes were wrongly assigned to the homozygote groups could account for these results. However, this would be unlikely to affect results from European populations and not Asian ones. Furthermore, the order of the 3 M subtype bands is M1, M3 and M2 after isoelectric focusing and so it is most unlikely that M1M2 would be incorrectly identified while M2M3 was correctly identified.

Thirdly, there could be differences in survival between individuals with the various PI M phenotypes. Since most of the subjects in the studies cited here would have been young adults, and mortality between birth and the age of 40 in European groups is now low, differences in survival between conception and birth may have to be invoked. Such differences could, in principle, be detected by testing neonates and their parents but this would require a large number of subjects.

At the other end of the age scale, a recent study in this laboratory [7] found that the frequencies of the various PI M subtypes in very old people, who had survived to the age of 85 years or more, were considerably different from those reported in younger people. This also suggests that PI M subtype has some effect on survival, although in the old subjects it was the M1 phenotype which was found in greater numbers than expected rather than the M1, M2 and M3 phenotypes which the literature reports suggest are all present in excess.

If the deviations from the expected frequencies are due to differences in survival, then they presumably reflect differences in PI function between M subtype homozygotes and heterozygotes. It is not easy to see how such differences could occur, because the two copies of any gene which each individual carries are generally expressed independently at the molecular level. Nevertheless, two reports [3, 4] have found quantitative differences in α_1-antitrypsin protein concentration and elastase-inhibitory capacity between PI M homozygotes and heterozygotes. Either M1M2 subjects [3] or both M1M2 and M1M3 subjects [4] had higher concentrations than would be expected from averaging the results of the relevant homozygotes.

In conclusion, the literature supports the view that, in European groups at least, the frequencies of PI M subtypes are significantly different from those expected, both in young adults and in the very old. How such a divergence from equilibrium is maintained, and what features of the PI lead to it, remain unknown at present.

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