Antiproteases and Down's syndrome in an Australian population*

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SUMMARY α_1 -antitrypsin phenotypes and α_2 -macroglobulin concentrations were determined for an Australian population of 286 Down's syndrome patients, 296 newborn babies, and 193 patients with non-specific mental retardation. There was no significant difference between the groups in the distribution of the phenotypes or level of heterozygosity. The phenotypic frequencies were 89.5% PiMM, 8.0% PiMS, and 1.0% PiMZ among controls. The mean α_2 -macroglobulin concentrations were investigated and it was shown that PiMZ phenotypes had a higher concentration, 2.67 ± 0.27 g/l (newborns) and 2.74 ± 0.32 g/l (Down's syndrome), in comparison with PiMS, PiSS, and PiMM.

The increased frequency of heterozygotes for α_1 -AT phenotypes among mothers of children with chromosomal aberrations noted by Aarskog and Fagerhol¹ suggested a relationship between α_1 -AT phenotypes and chromosomal abnormalities. This observation has been extended by Kueppers *et al*² who noted an excess of heterozygotes in the combined parental group as well as among patients with chromosomal abnormalities. Similarly, Fineman *et al*³ noted an increased frequency of heterozygotes among Down's syndrome patients in the older mothers groups; distribution of α_1 -AT phenotypes among parents were not determined. However, Arnaud *et al*⁴ were unable to observe any significant differences in the distribution of α_1 -AT phenotypes between Down's syndrome patients and controls.

In view of these contradictions, we report the results of investigations on α_1 -antitrypsin phenotypes as well as quantification of α_2 -macroglobulin in Down's syndrome, non-specific mental retardation, and in controls for an Australian population.

Materials and methods

α_1 -antitrypsin was phenotyped by isoelectric focusing in thin layers of polyacrylamide gels using the method of Kueppers,⁵ with minor modifications,

using LKB ampholines in the pH range of 4 to 6. The gel was prerun at a constant 25 mA for one hour. On completion of the run, the protein bands were precipitated in 10% trichloroacetic acid overnight and the gel washed in two rinses of distilled water. Proteins were stained using 0.25% amido black in 7% acetic acid and destained in 7% acetic acid. Rare phenotypes were identified by comparison with standards obtained through the courtesy of Dr M Fagerhol, Oslo, Norway.

QUANTIFICATION OF α_1 -AT AND α_2 -MG
Radial immunodiffusion was used to determine α_1 -antitrypsin and α_2 -macroglobulin concentrations in sera. The antisera were commercially available from Kallestad and Berhingwerke standard plasmas of known α_1 -antitrypsin and α_2 -macroglobulin concentrations (g/l) were used as controls. Samples for α_2 -macroglobulin levels were diluted 1:2 and samples for α_1 -antitrypsin levels were diluted in accordance with their phenotype. ZZ sera were used undiluted, SS, SZ, and MZ sera were diluted 1:10, and all others were diluted 1:20.

The plates were left at 4°C for 48 hours in the case of α_1 -AT and 72 hours for α_2 -MG and the resulting precipitin ring diameters were measured. A standard graph was constructed, using the control sera of concentration (g/l) vs the squares of the diameter of the precipitin ring. Results for the other samples were read off the graph using the same technique.

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TABLE 1 Phenotypic distribution of α_1 -AT in Australian populations

Samples	M	MS	MZ	GM	FM	FF	SS	SZ	Others	Total
Cord blood	89.5%	8.0%	1.0%	1.0%	—	—	1.0%	—	1 MN	296
Observed	265	21								
Expected	252.9	25.6								
χ^2_1	0.60	0.80								
Down's syndrome	88.8%	8.7%	1.4%	0.35%	0.35%	0.35%	—	—		286
Observed	254	25								
Expected	269.4	23.7								
χ^2_1	0.09	0.007								
Non-specific retardation	89.6%	8.7%	1.2%	—	—	—	0.5%	0.5%	1 MP	193
Observed	173	15								
Expected	181.4	16.0								
χ^2_1	0.4	0.005								
Pool	89.3%	9.0%	1.2%	0.5%	0.1%	0.1%	0.5%	0.1%	1 MN 1 MP	775
Observed	692	61	9							
Expected	728.5	65.6	8.7							
χ^2_1	1.8	0.30	0.01							

SAMPLES

Cord blood samples had been previously collected from the Mercy Maternity Hospital, Melbourne. Sera from patients with Down's syndrome and non-specific mental retardation were obtained as has been described by Ball *et al.*⁶ and Brackenridge *et al.*⁷

Results

The distribution of the α_1 -AT phenotypes is given in table 1. It is obvious that there is no significant difference in the frequency of the common phenotypes MM and MS in the three groups. The distribution of the two phenotypes MM and MS are found to be in Hardy-Weinberg equilibrium. The allele frequencies of Pi^m , Pi^s , and Pi^z are given in table 2. This table shows the differences in these three alleles between Australian, British, and American populations. Although the z allele frequency is considerably higher in the American population, the s allele frequency is lower. The allele frequencies for the Australian and British populations are very similar. The only difference in the distribution of α_1 -AT phenotypes is the occurrence of rare phenotypes GM, FM, and FF at a frequency of 1.05% among Down's syndrome patients. There was no difference in the frequency of

TABLE 3 Relationship between α_1 -AT phenotypes and α_2 -MG concentration

Phenotype	Source	No	α_2 -MG concentration (g/l)
MM	Cord blood	60	2.96 ± 0.56
MM	Down's syndrome	39	2.25 ± 0.50
MM	Adult blood donors	39	1.74 ± 0.41
MS	Cord blood	18	2.15 ± 0.45
MS	Down's syndrome	8	2.13 ± 0.56
MZ	Newborns	3	2.67 ± 0.27
MZ	Down's syndrome	3	2.75 ± 0.32
ZZ	Liver patients (children)	10	2.08 ± 0.28

total heterozygosity (11.5% among Down's syndrome, 10.9% among non-specific mental retardation patients, and 11% among newborn infants).

Table 3 shows the results of quantitative estimation of α_2 -macroglobulin and α_1 -AT in newborn children and in Down's syndrome. The mean α_1 -AT concentration among newborn children was 2.84 ± 0.88 g/l, while that of the Down's syndrome group was 3.06 ± 0.46 g/l. In contrast to this, however, the mean concentration of the α_2 -macroglobulin was 2.96 ± 0.56 g/l among newborn children, while it was 2.25 ± 0.50 g/l among Down's syndrome patients.

Discussion

The allele frequencies found in this Australian pooled sample compare very favourably with those of Cook⁸ for a British population. The allelic frequencies for Australian and British populations are much more similar than those of Rynbrandt *et al.*⁹ for an American population. This is not surprising when you consider the ethnic backgrounds of the countries involved.

Earlier investigations had shown an increased

TABLE 2 Distribution of Pi allele frequencies in selected populations

Allele	Frequency		
	Australian (this study)	British ⁸	American ⁹
Pi^m	0.940	0.950	0.930
Pi^s	0.045	0.043	0.034
Pi^z	0.006	0.007	0.0186

frequency of heterozygotes among patients with sex chromosome mosaicism. For example, Aarskog and Fagerhol¹ detected two MS among 14 patients (14%) which was higher than the 8 to 9% frequency of MS reported for Caucasian populations. However, these authors were unable to decide whether the increased frequency was among the paternal or maternal group or both. Kueppers *et al*² noted an increased frequency of heterozygotes among 21 patients with sex chromosome mosaicism (23.8%); this increase was noted in both the maternal and paternal group. Fineman *et al*³ observed that in Down's syndrome patients whose mothers were 35 and over at the time of their birth, there was an increased frequency of heterozygotes for α_1 -AT phenotypes.

The lack of an increase in heterozygosity for α_1 -AT variants in the current study contradicts the findings of Fineman *et al*.³ It may be argued that the discrepancy could be attributed to the lack of information regarding maternal age. This, however, is unlikely to provide a satisfactory explanation since a major proportion of Down's syndrome children are found among progeny of older mothers, while those of younger mothers constitute only a small proportion of the total.¹⁰ Similarly, Arnaud *et al*⁴ were unable to find an increased frequency of α_1 -AT heterozygotes among Down's syndrome children. Also, our sample sizes are considerably larger than those of Fineman *et al*,³ 286 as against 60 Down's syndrome patients.

Evans *et al*¹¹ reported differences in the relative concentrations of proteins in the electrophoretic bands 4 and 6, numbering being in order of decreasing anodal mobility. Among newborn children, the relative anodal bands, notably band 4, are more intense, the pattern being reversed after birth. The control population consisted of newborn children and a similar pattern was observed, even though isoelectric focusing rather than starch gel electrophoresis was used. These changes may represent developmental changes in the relative activities of multiple molecular forms of α_1 -antitrypsin. The significance of these changes is yet to be elucidated.

α_2 -macroglobulin, one of the antiproteases found in comparable concentrations in human serum, is capable of inhibiting trypsin in common with α_1 -AT. Therefore, it seemed worthwhile to study a wide variety of proteolytic enzymes, including the α_2 -macroglobulin concentration, among Down's syndrome patients as well as to investigate the possible influence of α_1 -AT phenotypes on α_2 -MG concentrations. α_2 -macroglobulin is age-dependent, with the concentration in cord blood varying between 2 and 4 g/l, which is 50% higher than the normal mean adult level.¹² The Down's syndrome

patients, mean age 40 years, were found to have a higher quantity of α_2 -MG which is in keeping with other published studies.^{13,14} The phenotypes MZ and MS did show differences from that of MM (table 3) and the SS phenotypes exhibited α_2 -MG levels similar to those of MS and MZ. In both newborn infants and Down's syndrome patients, the MS phenotype was lower in α_2 -macroglobulin concentration than the MZ phenotype. This may be the result of sample size effect, as the number of subjects in both the MS and MZ classes are small compared to the number in the MM classes. Since the frequency of ZZ phenotypes is very low among normal populations, we had no access to them among these patients and controls, so we used Pi_z homozygotes obtained from liver disease patients.

It is of interest to note that among ZZ phenotypes with liver disease the α_2 -MG level is considerably lower. This reduction is unlikely to be attributed to liver disease since α_2 -MG levels are reported to be raised among liver disease patients.¹⁵ The biological significance is, however, not clear at the moment. Twumasi *et al*¹⁶ have suggested that increased complexing of α_2 -MG to elastase in ZZ patients could exacerbate the tissue destruction by elastase. Although the proof is inconclusive, these trends suggest the possibility of interrelated regulations of antiproteases in the normal, and deviations in this balance between antiproteases and proteases could lead to disease. Future experimental work is designed to test some of these possibilities.

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