DETERMINATION OF ACETYLATOR PHENOTYPE WITH RESPECT TO ISONIAZID IN MALTA

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Introduction

The studies carried out involved isoniazid, an important chemotherapeutic agent in anti-tuberculous treatment. Two main aims were identified, and both were concerned with the existence of fast and slow acetylators (inactivators) of isoniazid. The first aim was the determination of the distribution ratio of fast and slow metabolisers in a sample of Maltese volunteers, this quite possibly being the first time any such attempt was made locally. A secondary design of the first aim was the possible correlation of factors, other than genetic ones, with the acetylator phenotype. The second aim was an attempt at reasserting certain genetic principles with regards to the isoniazid acetylator phenotype, in a Maltese context. These principles were established in 1960 by Evans et al and stated that the isoniazid acetylator phenotype is determined by a single pair of genes located on an autosomal chromosome, with the gene for slow acetylation recessive to that for fast acetylation.

Methodology

Determining the distribution ratio of the acetylator phenotypes in the Maltese islands

A group of one hundred and eighteen volunteers was selected for this study; certain inclusion/exclusion criteria were followed: all healthy individuals were initially deemed suitable for selection; those suffering from any disease which might have interfered with the absorption or metabolism of isoniazid, those with a history of drug allergy, or those on any concurrent drug therapy which might have interacted with the isoniazid were then excluded. Otherwise the volunteers were picked at random, with 62 males and 56 females being included. This methodology was based on a study by Raghupati Sarma et al (1976).

Each volunteer was given a 300mg oral dose of isoniazid, and urine was collected for the 5-6 hour period following administration. The volunteers were kept in a fasting state for two hours before and after ingestion of the isoniazid. The samples were analysed immediately or otherwise refrigerated to prevent loss of isoniazid due to degredation, since isoniazid is thermolabile.

The samples were then analysed to determine their isoniazid and acetylisoniazid content; the acetylisoniazid/isoniazid ratio (A/I ratio) of each individual was thus calculated. A volunteer was classified as a slow acetylator if his or her A/I ratio was less than 2. if the ratio was greater than 2 the volunteer was put down as a rapid acetylator.

The laboratory technique utilised in the determination of the isoniazid content was adapted from Venkataram et al (1972), Eidus et al (1971), and Eidus and Little (1961). Basically, the process involved adding sodium hydroxide and ammonium sulphate to a few ml of urine, shaking with a mixture of butanol and chloroform and then filtering.

The filtrate was then shaken with sulphuric acid, centrifuged and the supernatant collected. Two percent vanillin solution in alcohol was then added to the supernatant, and the solution examined spectrophotometrically at 380nm. The concentration of isoniazid was then determined by consulting a previously prepared standard curve for isoniazid.

The technique for the estimation of the acetylisoniazid content was modified from Eidus et al (1971) and Venkataram et al (1968). In brief, this method involved adding phosphate buffer, potassium cyanide, and chloramine-T to a sample of urine. The mixture was allowed to stand and after the addition of acetone, a spectrophotometric reading was taken at 550nm. The concentration of acetylisoniazid was found by once again consulting a previously prepared standard curve.

After determining the acetylator phenotype of all the volunteers the percentages of slow and fast acetylators in the sample were calculated. Statistical manipulations were also carried out to try and bring out any correlation between the acetylator phenotype and other factors such as age, sex, habits such as alcoholism, and weight.

Reworking the method of inheritance of the isoniazid acetylator phenotype in Maltese subjects

For the purpose of carrying out this aim, the acetylator phenotypes of 12 whole families were determined, utilising the same techniques as in the first study. Thirty-eight of the subjects were common to both studies. The frequencies of the dominant and recessive alleles were then calculated; this was done by taking the frequency of slow acetylators, assuming the gene for slow acetylation to be recessive then the frequency of the

recessive allele was the square root of the frequency of slow acetylators. Hence the frequency of the dominant allele was equal to one minus the frequency of the recessive allele.

The hypothesis that the gene for slow inactivation was then tested by comparing the observed matings with those expected by application of the Hardy-Weinberg law, and by comparing the numbers of expected and observed children of each phenotype. The same process was also carried out on the hypothesis that the gene for fast acetylation was recessive.

Results

The acetylator phenotype distribution and influencing factors

Table I illustrates the distribution of fast and slow acetylators in the random sample of volunteers taken from the Maltese population. Initially more volunteers were approached but these were deemed unsuitable for the purposes of the study for various reasons; for example, four had just been inoculated with the antituberculous vaccine, ten others were on concomitant drug therapy, and three others were suffering from stomach upsets which would have interfered with the absorption of isoniazid.

Table I:

Sex	Slow	Fast	Total
Male	32	30	62
Female	41	15	56
Total	73	45	118

Table II sums up the above results in terms of percentages; the fact that there were less females than males was accounted for by giving the female and male percentages equal weighting in the final percentage for the whole population. This was based on the assumption that the Maltese population is composed of equal numbers of males and females.

Table II:

Sex	Slow	Fast	Total
Male	51.6	48.4	100.0
Female	73.2	26.8	100.0
Total	124.8	75.2	200.0
Population Average	62.4	37.6	100.0

As regards the effect of other factors on the inactivator phenotype, age, the stage of physical fitness, alcohol intake and smoking habits were found not to have any significant bearing. It must be noted, however, that an amount of heavy drinkers did tend to be slow acetylators, but this figure was not large enough to be of statistical significance. Weight and sex were found to be correlated to the inactivator phenotype in the volunteers included in this sample.

The inheritance of the acetylator genotype

The acetylator phenotypes of all the members of each family were determined using the method described section 2.2. In all the number of volunteers participating in this study was 66, of which 37 were males and 29 were females.

The percentage of slow acetylators in the sample study on the distribution ratio of the acetylator phenotype was 62.4136%. in this case, the allele for slow acetylation was assumed to be recessive and the frequency of the recessive allele (Ir) was calculated by taking the square root of the frequency of slow acetylators. Thus the frequency of the recessive allele was calculated as the square root of 0.624136, that is, 0.7900 (=p). The frequency of the dominant allele for rapid acetylation was thus 1-0.7900, or 0.2100 (=q).

Table III:

Phenotypic Matings	Genotypic Matings	Expected frequency of Matings		Expected Occurrence	Observed Occurrence
S X S	$I_rI_r \times I_rI_r$	p^4	0.3895	4.67	5
R X S	$I_RI_R \times I_rI_r$	$2p^2q2$	0.0550		
	$I_Ri_r \times I_rI_r$	$4p^3q$	0.4142		
			0.4692	5.64	5
R X R	$I_RI_R \times I_RI_R$	q^4	0.0019		
	$I_RI_R \times I_RI_r$	$4q^3p$	0.0293		
	$I_RI_r \times I_RI_r$	$4q^2p^2$	0.1100	1.69	2
			0.9999	12.00	12

Table III displays the comparison of the observed and expected occurrence of the various possible types of matings for the hypothesis that the gene for slow inactivation is recessive. The results in Table III were analysed statistically and a value of 0.1528 was obtained for X_2. for 2 degrees of freedom this gave a value of p (this variable is in no way connected to the p utilised in the genetic argument above. it is simply a statistical variable) greater than 0.5 and this proved that there was no significant difference between the expected and observed results.

Table IV illustrates the comparisons made between the expected and observed phenotypes of children resulting from mating of couples whose phenotype was determined. The data fit the hypothesis that the phenotype for slow inactivation of isoniazid is represented by a homozygous recessive pair of genes, IrIr. Statistical examination of the results produced a value for X_2 of 1.05, and for 2 degrees of freedom this gave a value of p (this p is in no way connected to the p mentioned in previous genetic arguments; it is simply a statistical variable) greater than 0.5; this further substantiated the initial view that the difference between the expected and observed results was not significant. When the family data were examined in the same manner for the hypothesis that the gene for fast acetylation was recessive, the agreement of observed with expected results was found to be unsatisfactory.

Table IV:

Phenotypic matings	No. of matings	No. of children	No. of children of each phenotype			
			Fast		Slow	
			Exp.	Obs.	Exp.	Obs.
SxS	5	15	0	0	15	15
R x S	5	20	11.17	13	8.83	7
R x R	2	7	5.64	5	1.36	2
	12	42		18		24

Discussion/Conclusion

The results obtained satisfied the two initial aims of the studies carried out; the first aim of determining the distribution of the isoniazid acetylator phenotype for a sample of the Maltese population was achieved, with a 39.8/60.2 fast to slow ration being reported. The secondary designs of the first aim were also achieved, with no significant correlation being observed between acetylator phenotype and age, physical fitness, and smoking or alcohol intake. It must be noted, however, that people with a greater than average weight generally were slow inactivators; this may be due to a reduced metabolic rate in obese or overweight individuals, this being the result of hereditary influences or sedentary habit. As regards this second point no relation was found between acetylator phenotype and the state of physical fitness of the volunteers, as was already stated above. With reference to sex and the acetylator phenotype, male volunteers did tend to be more rapid inactivators, and the difference between the sexes was of some statistical significance.

The second aim of reworking the genetics proposed and the proven by Evans et al in 1960 was also satisfied. The results obtained, both as regards the numbers of the different types of matings and the numbers of slow and fast acetylator offspring agreed with the expected figures at high levels of statistical significance.

The value of the first aim of this dissertation was deemed partly as a statistical exercise, since it was the first time that the isoniazid acetylator ration has been determined utilising Maltese subjects, and partly as an investigation into the factors influencing the inactivator phenotype. The second aim of the dissertation was an exercise in genetics and proved that the methods applied for the inheritance of the isoniazid inactivator phenotype elsewhere, also apply to the Maltese population.

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