Amer J Hum Genet 25:327–331, 1973

Genetic Variation of Aryl Hydrocarbon Hydroxylase in Human Lymphocytes

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Aryl hydrocarbon hydroxylase (AHH) is one of the mixed function oxidases [1] in the microsomal fraction of many mammalian tissues. While the exact physiological role of this membrane-bound enzyme is not understood, it is known to hydroxylate a wide variety of exogenous hydrocarbons including many drugs, insecticides, steroids, and chemical carcinogens [2, 3]. The initial step in the microsomal oxidation of polycyclic hydrocarbons, catalyzed by AHH, involves the formation of epoxides [4] which are considered to be the active form of the carcinogen [5, 6].

The AHH enzyme is inducible, with an increase up to 10 times resting levels within 1–3 days after presentation of the inducing agent. A variety of substances act as inducers, including the hydrocarbon substrates [7], barbiturates, and others [8, 9]. Animal studies have demonstrated that extent of induction varies widely within a species [10]. Further, genetic studies of mouse strains suggest that the capacity for induction is controlled by a single autosomal locus [10, 11].

We recently demonstrated [12] that there is wide variation in extent of AHH inducibility in human lymphocytes. The present study was undertaken to determine if such variation is under genetic control.

SUBJECTS AND METHODS

Subjects

Subjects were normal, healthy volunteers ranging in age from 2 to 89 years. The total number of subjects was 353. Included were 67 families with 165 children (86 males and 79 females). Also included in the families were 12 sets of identical twins, five sets of fraternal twins, two sets of identical triplets, and one fraternal triplet.

Methods

Leukocytes were cultured as previously described [12, 13] in modified Eagle's minimal essential medium (Gibco) supplemented with fetal calf serum (20%), phytohemagglutinin (1%), pokeweed mitogen (1%), and heparin (60 units/ml). The culture tubes contained approximately three million cells each in 5 ml medium. After incubation for 72 hr at

Received January 30, 1973.

This work was supported in part by research grant GM 15597 from the National Institutes of Health and grant Ke 217/1 Deutsche Forschungsgemeinschaft (DFG).

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37°C, the cells received 5 μl of 1.5 mM 3-methylcholanthrene (3-MC) in acetone for an additional 24 hr and were assayed for AHH activity by a modification of the method of Nebert and Gelboin [14].

The specific activity is expressed in units per 10^6 cells (10^6 cells contain 0.15–0.20 mg protein). One unit of aryl hydrocarbon hydroxylase activity is defined as that amount of enzyme catalyzing the formation per minute at 37° C of hydroxylated product with fluorescence equivalent to that of 1 pmole of 3-hydroxybenzo(a)pyrene [15]. All values were corrected for recoveries (60%–65%) and represent the average in duplicate samples.

RESULTS AND DISCUSSION

The extent of induction is expressed as the ratio of activity after induction over that present before induction. Figure 1 shows the distribution of the levels of inducibility in the population studied. Note that there are three peaks, the largest at a low inducibility level, a smaller peak at an intermediate level, and the smallest at a high inducibility level. On the basis of these results, the subjects were divided into three groups, designated low, intermediate, and high inducibility, with the cutoff points as indicated by the arrows on figure 1 (i.e., 2.5 and 3.6). Even the lowest subjects showed some inducibility, which is contrary to the findings in mice where some strains showed zero inducibility [10, 11].

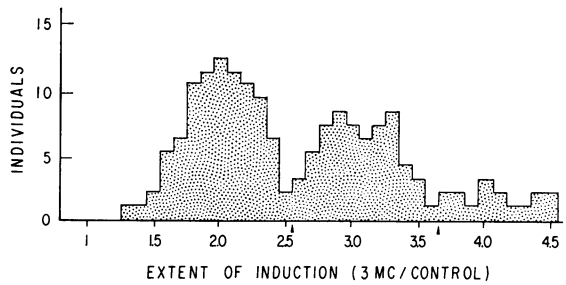

FIG. 1.—Distribution of inducibility levels. Extent of induction expressed as the ratio of AHH activity after induction with 3-methylcholanthrene by activity before induction.

Assuming a single-locus control of this enzyme and designating the two alleles AHH^a and AHH^b with the respective phenotypes being AA, AB, and BB (low, intermediate, and high inducibility), the distribution is in agreement with the Hardy-Weinberg equilibrium. Frequencies of the alleles AHH^a and AHH^b are .717 and .283 (table 1).

TABLE 1

AHH PHENOTYPES AND ALLELE FREQUENCIES

	PHENOTYPE			ALLELE			
	AA	AB	BB	AHH^a	AHH^b	χ^2	P
Observed	86	59	16	.717	.283	1.48	$.50 > P > .30$
Expected	82.8	65.3	12.9	...	...	...	...

NOTE.—$N = 161$.

In obtaining these data, emphasis was on extent of induction rather than on absolute levels of activity either at resting or at the induced stage. This was primarily because the genetic studies reported in the literature on animals emphasize inducibility. The three groups were distinguishable only at the induced levels of activity ($P < .001$). Base levels alone could not distinguish these groups ($P > .05$). However, there was a high correlation within each group between base and induced levels (correlation coefficients .80, .89, and .91 for the low, intermediate, and high groups, respectively).

In order to check the stability of inducibility we tested several subjects repeatedly under different culture conditions but with the same inducing agent over a period of 6 months. The deviation in extent of induction ranged between 5% and 10% which coincided with the mean error in our test system.

In figure 2 we plotted the resting against the induced levels. The three types of

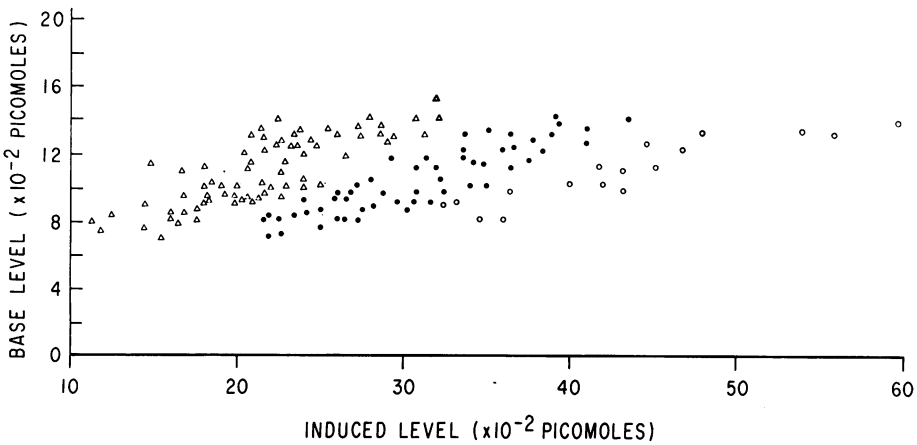

FIG. 2.—Scattergraph comparing uninduced with induced levels of AHH. Triangles represent subjects in the low-inducibility group, filled circles are the intermediate, and open circles are the high-inducibility subjects.

marks employed indicate whether the individual is a low, intermediate, or high inducer. The three groups are quite separate and indicate a direct relationship between resting and induced levels of activity. But as mentioned above, the base levels alone could not distinguish the three groups.

In the family studies, all possible crosses were found as shown in table 2. The distribution of phenotypes in the children reveals no deviation from the phenotypes expected. The results of the twin study are in good agreement with these findings, and the deviation in extent of induction between identical twins or triplets was less than 10%. There was no significant sex difference ($P > .20$) in magnitude of induction.

The cited reports on variation in inducibility among strains of animals [11] has led to a hypothesis that a regulator locus was involved in the genetic variation. However, the correlation between resting and induced levels within the three groups

TABLE 2

FAMILY STUDIES

MATING TYPE	N	TOTAL NO. CHILDREN	PHENOTYPES		
			AA	AB	BB
AA × AA	17	39	39	...	...
AA × AB	28	63	31	32	...
AA × BB	4	10	...	10	...
AB × AB	10	35	9	17	9
AB × BB	6	13	...	9	4
BB × BB	2	5	...	...	5
Total	67	165	79	68	18

suggests that a structural gene is involved, with the two forms of the enzyme having different specific activities and with both alleles being inducible. Further studies of the purified enzymes will be required to answer this question.

Whether the AHH of lymphocytes is representative of this enzyme activity in other tissues has not yet been determined and is the subject of a present study. Significance of the enzyme to susceptibility and resistance to cancer in man is not yet known. However, recent studies from this laboratory have indicated that AHH is present in pulmonary alveolar macrophages and is significantly induced by inhalation of cigarette smoke. The amount of such inducibility appears to vary among individuals and to correlate with the values obtained in their cultured lymphocytes [16].

Tokuhata [17] demonstrated that susceptibility to lung cancer is inherited by showing excess mortality risk in both smoking and nonsmoking relatives of probands. This may be of direct pertinence to these findings. Since there is strong suggestion that epoxides are the ultimate carcinogenic form of polycyclic hydrocarbons, their carcinogenic activities may be determined by the relative activities of the epoxide-forming enzymes and epoxide-degrading enzymes [18]. Further studies are underway to determine the relationship between extent of induction and susceptibility to lung cancer.

SUMMARY

Aryl hydrocarbon hydroxylase (AHH), an enzyme involved in metabolism of chemical carcinogens, was assayed in cultured human lymphocytes. Both constitutive and induced levels were determined, induction being effected by 3-methylcholanthrene. The sample was comprised of 353 healthy subjects including 67 families with 165 children. Inducibility varied widely, ranging from 1.3 to 4.5 times resting levels. The population separated into three groups, low, intermediate, and high inducibility. The results suggest a single locus of genetic control, with gene frequencies of the low and high alleles being .717 and .283, respectively. All six possible mating types were obtained and the expected offspring were found. Resting

levels of AHH also varied, but within a much smaller range. However, resting and induced levels were highly correlated.

Implications of AHH activity for susceptibility and/or resistance to certain cancers are being investigated.

ACKNOWLEDGMENT

We are most appreciative of Dr. William J. Schull's help with the statistical analysis.

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