A Genetic Study of β-Aminoisobutyric Acid Excretion[1]

JEAN DE GROUCHY* AND H. ELDON SUTTON

Department of Human Genetics, University of Michigan Medical School, Ann Arbor, Michigan

INTRODUCTION

SINCE THE simultaneous discovery by Crumpler, Dent, Harris, and Westall (1951) and by Fink, Henderson and Fink (1951) that a particular amino acid, β-aminoisobutyric acid (BAIB), may be excreted in large quantities in the urines of certain individuals, several investigators have shown this trait to be characteristic of individuals and largely independent of environmental factors. Harris (1953), using two-dimensional paper chromatograms, classified individuals as "excretors" or "non-excretors" when the intensity of the spot formed for BAIB was visually greater or less than the intensity of that formed for alanine. On this basis he found that the frequency of "excretors" was 9.6 per cent in the United Kingdom and suggested that the trait was due to a single recessive gene. These findings were confirmed by Calchi-Novati and co-workers (1954) in an Italian population. Gartler (1956), although using a more quantitative determination of the excretion of BAIB, namely by measuring the transmission of light through the spot on paper chromatograms, also classified individuals as "excretors" and "non-excretors" in his genetic analysis of BAIB excretion. He concluded that the frequency of excretors was less than 2 per cent and that the inheritance of the trait was compatible with a single recessive gene hypothesis.

The above studies have been faced with the difficulty of studying a trait which is relatively rare in Western European populations. The discovery by Sutton and Clark (1955) that the frequency of excretors is considerably elevated in populations of Mongoloid extraction offers an excellent opportunity to attempt to elucidate the genetic mechanisms involved. Hence, this paper will be primarily concerned with a genetic study of families drawn from a Chinese and Japanese population.

METHODS

The subjects of the present study are individuals of Chinese and Japanese ancestry, most of whom were born in the Orient but who are residing at present in Ann Arbor, Michigan. Among the 91 subjects studied seventeen families are included, 30 children having been produced from these matings. The small number of children reflects the fact that the parents for the most part are still young. No evidence of a difference between Chinese and Japanese with respect to BAIB excretion has been found.

The experimental procedures employed were the same as reported earlier from this laboratory (Sutton and Clark, 1955) and are based on those used by Berry, Sutton, Cain and Berry (1951). One to three first morning urine samples were col-

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* Attaché de Recherches au Centre National de la Recherche Scientifique, Paris, France. Recipient of a Fellowship from the Rockefeller Foundation.

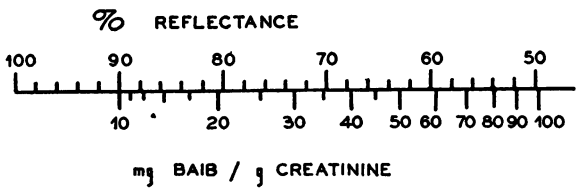

FIG. 1. Scale for conversion of reflectance readings (% R) into mg β-aminoisobutyric acid per g creatinine.

lected from each subject and kept frozen until ready for analysis. Two dimensional paper chromatograms were prepared with an aliquot of urine containing 50 micrograms of creatinine and were resolved with phenol and lutidine as the two solvents.[2] The amino acids were made visible by dipping the dried chromatogram through a 0.2 per cent solution of ninhydrin in 95 per cent ethanol, followed by heating for 7 minutes at 95°C. The heating is essential since BAIB, unlike many other amino acids, does not react appreciably with ninhydrin at room temperature.

The amount of the amino acid present was estimated by measuring the reflectance of the colored spot with a Beckman Model B reflectance attachment, using a colorless portion of the chromatogram to adjust the instrument to 100 per cent reflectance. Throughout the present report the reflectance values (R) are used. It will be seen from the conversion scale in Figure 1 that the reflectance scale is a logarithmic transformation of the absolute values. An R of 100 corresponds to no excretion and an R of 50 to very high excretion.

RESULTS AND DISCUSSION

The independence of BAIB excretion of age and sex has been observed by other investigators in populations with a low frequency of excretors (Harris, 1953; Gartler, 1956). In a group of 69 individuals of Oriental extraction studied in this laboratory the coefficient of regression of BAIB excretion on age was found to be −0.022, which is not significantly different from zero. The age range included was 1 month to 60 years. Likewise, no association with sex was detected.

That diet is of little importance in determining BAIB excretion is demonstrated by the absence of significant correlation ($r = -0.208$) between the 17 husband and wife pairs, many of whom were high excretors. This again is consistent with results obtained by other investigators.

The existence of naturally occurring discrete categories of individuals has been a matter of considerable discussion and conjecture. Gartler (1956) was unable to detect any bimodality in his population, but so few high excretors were present that the chances of demonstrating bimodality were small. In Figure 2 the population distribution is shown for the Japanese and Chinese populations which we have studied. Since the 30 children are excluded none of the individuals represented is related. Although there are a large number of high excretors present, no evidence is provided that individuals fall into discrete categories. It would seem necessary, therefore, in any genetic analysis of BAIB excretion to treat the excretion rate as a continuous variable. It is interesting to note that in 64 individuals from whom three urine samples were obtained and whose mean R fell between 86 and 44 the average stand-

[2] In the case of very young children whose creatinine excretion cannot be used as a measure of urine dilution, it has been found that 50 microliters of urine is a suitable test quantity.

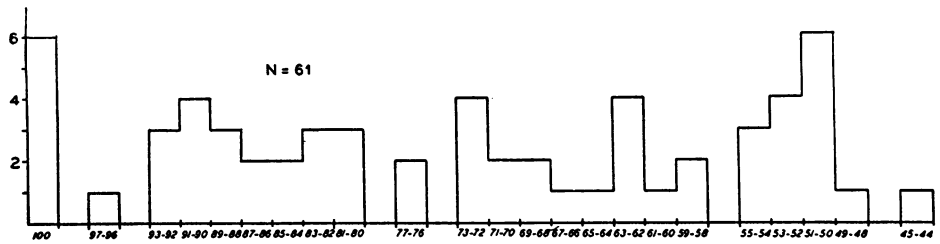

FIG. 2. Distribution of R values for β-aminoisobutyric acid excretion in a population of Chinese and Japanese ancestry.

ard deviation within samples from one individual is 3.28 R units. This indicates the degree of individuality which characterizes this trait.

Among the Chinese and Japanese groups which we have examined were 17 families with one or more children. These have been studied in hope of obtaining addi-

TABLE 1. URINARY EXCRETION OF β-AMINOISOBUTYRIC ACID IN 17 FAMILIES OF ORIENTAL EXTRACTION

Family Number	R* Father	R Mother	Mid-Parent	R Offspring	Sex	Age in Years	Mean Offspring
1	97	53	75	65	M	6	65
2	94	68	81	87	F	9	87
3	87	85	86	92	M	8	89.7
				86	F	7	
				81	F	5	
4	68	97	82.5	85	M	5	85
5	82	79	80.5	73	M	7	73
6	80	71	75.5	88	M	½	88
7	58	82	70	71	M	7	66
				61	F	6	
8	65	62	63.5	62	M	17	62
9	57	60	58.5	51	F	12	54
				59	F	10	
				52	F	4	
10	71	92	81.5	41	M	2	41
11	91	83	87	100	M	2	100
				100	F	5	
12	84	63	73.5	64	F	5	59
				54	M	2	
13	58	93	75.5	86	F	5	79
				81	F	11	
				84	F	8	
				65	F	3	
14	68	81	74.5	65	M	30	66.6
				67	F	29	
				68	F	11	
15	80	50	65	53	F	8	48
				43	M	5	
16	51	73	62	90	M	4	90
17	100	80	90	81	M	3	81

* R = Per cent reflectance value obtained from two-dimensional chromatograms.

tional information on the genetic mechanisms operating in BAIB excretion. In Table 1 are grouped for each family the following information: excretion rate of both parents, mid-parent value, excretion rate, age and sex of each offspring as well as a mean sibship value. As already pointed out the trait has been found to be independent of age and of sex.

A three-dimensional representation of our family data is shown in Figure 3. Sibs are united by a horizontal line and the length of the vertical segments is proportional to the actual excretion of BAIB (e.g. a long segment corresponds to a high excretor). In the area which corresponds to low excreting parents only low excreting children appear. Conversely the mating of high excretor by high excretor—referring to a range rather than to an attribute—results in a high excretor child. Also, one may notice in sibships of two or more that the values of sibs are similar, thus showing little tendency for segregation.

Further information is provided by measuring the correlation between parents and offspring. The mid-parent/all offspring correlation coefficient is 0.531 while the mid-parent/mean offspring correlation coefficient is 0.463. Although these coefficients are obtained from a different number of "points" on the diagram, namely, 30 in the first case and 17 in the second, the number of degrees of freedom should be chosen in both cases as $17 - 2 = 15$. Thus the mid-parent/all offspring correlation coefficient is significantly different from zero at the 0.03 level and the mid-parent/mean offspring at the 0.06 level. The fact that both coefficients are not significantly different from each other reflects the small amount of segregation mentioned earlier.

In an attempt to detect any paternal or maternal predominant effect, correlation coefficients have been computed for each parent separately. r (father/all offspring) = 0.273 with d.f. = 15 is non-significant, while r (mother/all offspring) = 0.518 with d.f. = 15 is significant at the 0.03 per cent level. The reality of this difference may be tested by computing partial coefficients of correlation. Then r (father/all offspring, holding mother constant) = 0.543, d.f. = 14, and r (mother/all offspring, holding father constant) = 0.652, d.f. = 14. These coefficients show no significant difference from each other, indicating no demonstrable maternal effect.

Finally, if the trait is genetically determined, we may expect a higher variance between sibships than within sibships. Unfortunately the number of sibships larger than one is small, only eight such sibships are available in our data. Nevertheless, these families give good evidence of a very much higher variance between than within sibships ($F = 17.56$, d.f. = 7, 13, $P < 0.001$).

The analysis of our data as well as the results published by others support the

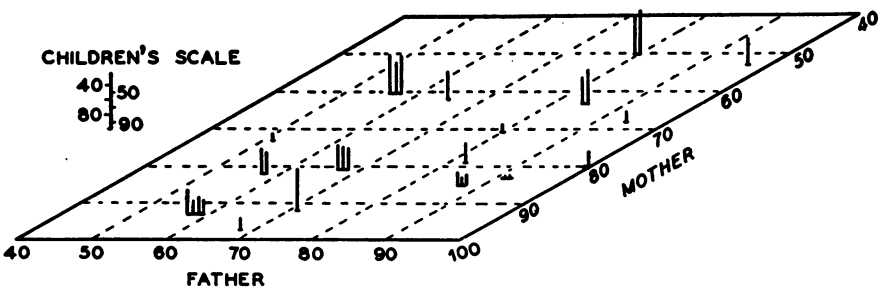

FIG. 3. A three-dimensional diagram of β-aminoisobutyric acid excretion in 17 families of Chinese and Japanese ancestry.

hypothesis of genetic determination of the trait. The correlation that exists between parents and offspring in the absence of a correlation between parents, may only be explained by a genetic mechanism. A difficulty arises, however, when one attempts to elucidate the mode of inheritance. As we have pointed out we find no evidence for classifying individuals into discrete categories which would allow us to test the hypothesis of a single recessive gene.

On the contrary, the fact that the trait is observed to be a continuous variable is hardly compatible with the hypothesis of a two-allele system acting independently of other genetic or environmental factors. The segregation observed in a few families in these and other data (Gartler, 1956) suggests, however, that the major portion of the variation may be produced by the activity of a small number of genes.

In the present study, the subjects have been drawn from a normal population of Orientals. It should be borne in mind that the excretion of BAIB has not been proved to result from identical mechanisms in all individuals. For example, the reported association of BAIB excretion with various pathological conditions should not, on a priori grounds, be assumed to be of identical nature.

SUMMARY

From a study of families of Oriental extraction, support is given to the hypothesis of genetic determination of urinary excretion of β-aminoisobutyric acid. However, the data do not support the hypothesis of a two-allelic system acting independently of other genetic or non-genetic factors. The variation observed may result from a small number of genes.

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