*HEPATITIS AND AUSTRALIA ANTIGEN: AUTOSOMAL RECESSIVE INHERITANCE OF SUSCEPTIBILITY TO INFECTION IN HUMANS**

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Abstract.—Examples of inherited susceptibility to infection controlled by genes segregating at one or a small number of loci have been identified in lower animals. In this study we report data on what appears to be a similar situation in humans. "Australia antigen" is an antigen found in the sera of patients with acute and chronic hepatitis, and it may actually be a form of virus. It is very common in many tropical areas, and people in these areas having the antigen appear to be hepatitis carriers. The antigen is detected by immunodiffusion in agar gel (Ouchterlony method). Individuals with the antigen are designated Au(1) and those without it Au(0). Family studies involving 1797 different individuals residing on the island of Bougainville are consistent with the hypothesis that susceptibility to chronic infection with the "antigen" is controlled by an autosomal recessive gene (Au^1). This confirms the conclusions previously arrived at from similar (but less extensive) studies on the island of Cebu. Individuals with this inherited susceptibility do not ordinarily have overt manifestations of hepatitis.

The sign test was used to determine family clustering. Segregation analysis was performed by the method of C. A. B. Smith. In the 41 Au(0) × Au(0) matings, 53.8 recessives were expected in the offspring and 56 were seen ($0.7 > p > 0.5$). In the Au(1) × Au(0) matings, 40.1 recessives were expected and 42 were seen ($0.7 > p > 0.5$). The Au(0) × Au(0) matings were also analyzed by the method of Li and Mantel, in which the recessive ratio of 0.25 is expected by the genetic hypothesis. The values observed were 0.2527 for the Bougainville study and 0.2461 for the Cebu study.

Inherited resistance and susceptibility to infection has been described in several animal species. There is a single gene-controlled factor which prevents infection of mice by a group of related viruses including yellow fever, West Nile fever, Japanese B encephalitis, and others but does not prevent infection with other viruses.[1] Gowen[2] has described inherited resistance to bacterial infection in mice and has emphasized the specific nature of the resistance which develops. There is evidence for inherited susceptibility to several forms of virus-induced neoplasms in animals. These include polyoma virus in mice,[3] in which two or three independent genes may be involved in the determination of resistance;[4] mammary tumor in mice;[5] leukemia in mice, where the inherited factor appears to be related to the histocompatibility locus;[6] Rous sarcoma;[7] fowl leucoses;[8] and others. In humans, several inherited diseases carry with them an increased susceptibility to infection; for example, patients with the

inherited disease sickle-cell anemia are unusually susceptible to *Salmonella* infections, with osteomyiletis as a frequent complication, while the heterozygotes appear to have an increased resistance to falciparum malaria. Patients with inherited sex-linked agammaglobulinemia are highly susceptible to several bacterial infections. There is also evidence for differences in infectious disease susceptibility between different population groups (i.e., increased tuberculosis infection in American Indians and increased infection with coccidioidomycosis in Filipinos and American Negroes) and some of this difference in susceptibility may be inherited. These have been reviewed recently.[9-11]

In this paper we will describe family studies of a serum factor closely associated with acute and chronic viral hepatitis. The segregation of the trait in the families is consistent with simple recessive autosomal inheritance, and the data can be interpreted as an example of increased susceptibility to a chronic virus infection in humans, apparently controlled by genes segregating at a single autosomal locus.

In 1966 we reported on the family clustering and segregation of a serum antigen present in high frequency in certain tropical populations.[12] Because it had been first identified in an Australian aborigine, the antigen was termed "Australia antigen." In the family study we utilized data collected on the Visayan island of Cebu in the Philippines. In the 53 families studied, the antigen was more commonly found among relatives of individuals who had the antigen (17%) than among the relatives of those who did not have it (2%). The hypothesis of simple autosomal recessive inheritance was tested in the families in which the trait was present. (Inspection of pedigrees ruled out simple autosomal dominant and sex-linked inheritance.) Using the segregation analysis method of C. A. B. Smith[13] (Table 3), we found a very close fit to the numbers predicted by the hypothesis; the data provided strong support for the hypothesis of simple autosomal recessive inheritance.

This initial evidence for the genetic determination of the presence of the antigen was interesting since we know that Australia antigen also has features of an infectious agent associated with hepatitis.[14-17] This has been reviewed[16, 17] and will be summarized here. Since 1961 we have systematically examined the sera of transfused patients for the presence of precipitating isoantibodies against constituents of human sera.[18] With these antisera, the Ag system (inherited antigenic specificities on the low-density lipoproteins)[19] was discovered. We subsequently found that patients who received large numbers of transfusions (particularly those with hemophilia) quite often develop specific antibodies against Australia antigen.[20-22] Antisera may also be produced by immunizing rabbits with the serum of a patient with Australian antigen and absorbing with a serum which does not contain the antigen.[23]

The antigen occurs in high frequency in acute hepatitis,[14-18] but in most cases it is transient (days or weeks). The association of hepatitis with Australia antigen has been confirmed by Okochi and Murakami,[22] Prince[24, 25] (the SH antigen of Prince is indistinguishable from Australia antigen), and Vierucci,[26] with reference sera from our laboratory. It also occurs chronically (for months or years) in Down's syndrome patients,[14, 27] in three forms of leukemia,[14, 21] and

in apparently normal people in vast areas of Asia and Oceania as well as elsewhere in the tropics.[12, 20] In these groups of patients it appears to be associated with chronic anicteric hepatitis.[22, 28, 29] The liver abnormality may be very slight, particularly in the case of the apparently normal tropical populations referred to.

As noted, Australia antigen is more common in the general populations in some tropical countries (6–25 %) and in Japan (1%) than it is in the United States (0.1%). The studies by Okochi and Murakami[22] in Japan show that Australia antigen may be transmitted by transfusion. Patients transfused with the blood of donors containing Australia antigen may develop the antigen in their own blood, along with hepatitis, or they may develop antibodies to Australia antigen with no evidence of disease. Hence, the detection of the antigen in donor bloods is useful in screening for hepatitis carriers. It has also proved very useful in detecting occult cases of hepatitis in patients with renal disease receiving chronic hemodialysis.[30] The "Au test" is now being used for the diagnosis of viral hepatitis and for the detection of hepatitis carriers.

Studies on the Down's syndrome patients demonstrate that both environmental (infectious) and host (congenital) factors are operating to determine the presence of the antigen.[17, 29] In large institutions, Australia antigen is very common in Down's syndrome patients but extremely rare in other mentally retarded children and in normal individuals. That is, there is a host factor in the Down's patients which makes them more susceptible to chronic infection with Australia antigen. Australia antigen was not found in Down's patients who live at home and attend day schools. Down's patients who are in-patients at small private institutions where sanitation and care are generally better have extremely low frequencies (3%) of the antigen. This is compatible with the explanation that some environmental factor, presumably an infection, is also operating and that the crowded conditions of large institutions lead to much higher frequencies of the antigen.

Australia antigen can be isolated by centrifugation in high-density sugar gradients. Isolated fractions examined under the electron microscope contain large numbers of particles of approximately 200 Å diameter;[31] this is about the size postulated for hepatitis virus. Although the appearance of the particles is consistent with that of a virus, this, of course, does not represent firm evidence that these particles are in fact virus.

All these data taken together (and briefly reviewed here) have led to the hypothesis that Australia antigen is or is associated with an infectious agent that occurs in viral hepatitis. The theoretical consequences of this will be discussed elsewhere.[17]

In view of these findings, it became extremely important to retest the genetic hypothesis by further family studies. Collections of sera on the island of Bougainville, Trust Territory of New Guinea, have made this possible. This paper contains a report of these studies.

During 1966–1967 one of us (J. F.) collected blood from individuals in 18 villages on Bougainville. In each village he attempted to collect blood from as large a number of individuals as possible. About 85% of the people over two

years old were included in the collection. The majority of these sera were made available for Australia antigen testing. These populations are described in detail elsewhere.[32] A total of 1797 sera were tested, including 617 family groupings. ABO and Rh blood groups and serum haptoglobin types were available in the individuals whose serum had been tested. If the paternity of any of the offspring in a family was questioned by the use of these markers, then the entire family was removed from the calculations and data from these individuals do not appear in the tables given herein. The presence or absence of the antigen in the plasma was determined by immunodiffusion in agar gel by a micro-Ouchterlony method.[33] In this, the antiserum against Australia antigen is placed in the center well of the Ouchterlony pattern and the sera to be tested in the peripheral wells. The presence of the antigen is indicated by the appearance of a precipitin line which stains red with azo carmine. Human antiserum from a hemophilia patient[21] and an antiserum made by the immunization of a rabbit[23] with a serum containing Au(1) were used. The sera to be tested were scored as Au(1) if a precipitin band was seen under these experimental conditions and as Au(0) if a precipitin band was not seen. After the sera were scored for the presence or absence of antigen, results were recorded on the pedigree charts. In this manner the individuals testing the sera were not aware of the family relationship and the scoring was objective.

Results and Discussion.—The frequency of Au(1) in the 18 villages varied from 2.9 to 23.9 per cent. In order to determine if there is family clustering for the trait, IBM punch cards were prepared for each of the 1797 sera, given sequential numbers, and sorted in the IBM 1620 with a random numbers program. The cards were then examined in turn until the card of an individual who was scored as Au(1) was found. The next card of an individual of the same sex and within five years of the same age, but who was scored as Au(0), was selected as a control. The cards of the immediate family (mother, father, daughter, son, brother, sister, grandmother, grandfather, grandson, granddaughter) of the individual scored Au(1) were then examined and the frequency of Au(1) was determined. The same was done for the family of the control (Au(0)) individual. Twenty-four pairs were studied in this manner.

The significance of the results was tested by the Sign test.[34] If the frequency of Australia antigen was higher in the family of the Au(1) individual than in that of the control (Au(0)), it was scored as positive; if the reverse was true, as negative. If there was no clustering in the families of individuals with Australia antigen, then an equal number of positives and negatives would be expected; but this was not seen (Table 1). Twenty-one of the twenty-four pairs studied were positive, two were negative, and one was the same; and this is highly unlikely to be due to chance ($p < 0.001$). This difference was not due to the differences in Au(1) frequencies in the villages from which the case and controls were selected.

Inspection of the pedigrees indicated that if the trait were inherited, it could not be inherited as a simple autosomal dominant trait or as a sex-linked trait. A simple autosomal, recessive hypothesis, the same as that tested in the Cebu material,[12] was also tested with the present material. This hypothesis states

TABLE 1. *Clustering of Au(1) in families as shown by the Sign test.*

1	2	3	4	5	6	7	8	9	10
Au(1)		Au(0)			Au(1)		Au(0)		
Family members	Au(1), %	Family members	Au(1), %	Sign	Family members	Au(1), %	Family members	Au(1), %	Sign
14	14.7	7	14.7	±	8	37.5	18	16.6	+
3	66.6	11	0	+	33	9.1	24	8.3	+
13	15.4	14	0	+	12	25.0	14	14.7	+
9	22.2	8	0	+	10	10.0	19	31.6	−
12	50.0	10	10.0	+	17	29.4	29	0	+
17	41.1	22	13.5	+	21	9.5	3	0	+
10	20.0	11	0	+	21	9.5	13	0	+
5	40.0	8	37.5	+	33	21.2	13	0	+
21	10.5	8	0	+	27	25.8	16	12.5	+
14	50.0	8	0	+	9	44.4	15	0	+
11	27.2	34	20.6	+	9	11.1	13	0	+
15	6.7	10	30.0	−	17	11.7	13	0	+

The number of family members and the per cent with Au(1) in the family of Au(1) individuals are given in columns 1, 2, 6, and 7. The same data for individuals who are Au(0) are given in columns 3, 4, 8, and 9. When the percentage of Au(1) was greater in the families of the Au(1) individuals than it was in the families of the Au(0) individuals, the doublet was scored as positive; if the opposite was the case, as negative. There were 21 positives and 2 negatives; it is highly unlikely that this is due to chance ($p << 0.001$).

that in individuals homozygous for a gene designated Au^1 (i.e., genotype Au^1/Au^1) Australia antigen would be detectable by the Ouchterlony method (phenotype Au(1)). In individuals homozygous for the alternate gene (Au/Au) and heterozygotes (Au^1/Au), Australia antigen (phenotype Au(0)) would not be detectable.

Two methods of segregation analysis were used to test the genetic hypothesis. In Smith's method,[13] the Au(0) × Au(0) and Au(1) × Au(0) matings are considered separately and corrections are made for family size.

For the Au(1) × Au(0) matings the calculations of Smith's Table 3 are followed, and the results are shown here in the upper part of Table 2. If the genetic hypothesis is correct, then 40.1 recessives are expected, and 42 are seen. The variance (as defined by Smith) is 11.0, the $\chi^2 = 0.341$ and $0.7 > p > 0.5$. This is a good fit to the expected. Included in this calculation are ten families in each of which one parent was positive and the other untested. It was assumed in each case that the untested parent was Au(0). The probability of a positive by positive mating is about 1/100 in this population.

The calculations for the Au(0) × Au(0) matings, which follow those of Smith's Table 6, are given here in the lower part of Table 2. If the genetic hypothesis is correct, then 53.8 recessives (i.e., Au(1)) are expected, and 56 are seen, a very close fit ($\chi^2 = 0.412$, $0.7 > p > 0.5$). Included in this calculation are eight families with at least one positive offspring in which the phenotype of one parent was Au(0) and in which the other parent was not tested. It was assumed in each case that the untested parent was Au(0). In this population the chance of an untested parent's being Au(1) is about 1 in 10; hence it is unlikely that more than one has been misclassified. If families in which neither parent was tested are also included, then the values for expected and observed recessive children are 77.8 and 82 with $\chi^2 = 1.044$. This is also a good fit. The results are summarized in Table 3.

TABLE 2. *Segregation of Au(1) in families with at least one Au(1) child.*

Number of children in family	Number of families	Recessives: Observed	Recessives: Expected	Variance
	Mating type: Au(1) × Au(0)			
c	m_c		$m_c a_c$	$m_c b_c$
1	6	6	6.000	0
2	5	5	6.665	1.110
3	4	8	6.856	1.960
4	6	17	12.798	4.692
5	3	6	7.743	3.246
Totals	24	42	40.062	11.008
	$\chi^2 = 0.341$	$0.7 > p > 0.5$		
	Mating type: Au(0) × Au(0)			
c	M_c		$M_c A_c$	$M_c B_c$
1	4	4	4.000	0
2	13	15	14.859	1.586
3	13	16	16.861	3.419
4	4	8	5.852	1.680
5	5	9	8.195	2.960
6	1	2	1.825	0.776
8	1	2	2.223	1.172
Totals	41	56	53.815	11.593
	$\chi^2 = 0.412$	$0.7 > p > 0.5$		

Computations by the method of Smith[13] as per Tables 3 and 6 of his paper. There was one family not included in the calculations in which the mother was positive and all her seven children were positive but serum was not obtained from the father. We are attempting to test this parent.

TABLE 3. *Summary of calculations by Smith's[13] method for Cebu and Bougainville families, showing variance and χ^2 (1 degree of freedom).*

Location	Type of mating	No. of families	Recessives: Observed	Recessives: Expected	Variance	χ^2
Cebu	Au(1) × Au(0)	7	12	12.189	3.556	0.010
Cebu	Au(0) × Au(0)	24	33	32.705	8.069	0.010
Bougainville	Au(1) × Au(0)	24	42	40.062	11.008	0.341
Bougainville	Au(0) × Au(0)	41	56	53.815	11.593	0.412

The segregation analysis method recently introduced by Li and Mantel[35] was also used to test the genetic hypothesis in the Au(0) × Au(0) families. In this, the total number of children t who are the issue of Au(0) × Au(0) matings, the number of recessives r (i.e., Au(1)) among these, and the number of children j who are the only recessive offspring in the family are determined. The proportion

$$p' = \frac{r - j}{t - j}$$

is 0.250 for an "ideal" recessive trait. Li and Mantel[35] give the rationale for this calculation and show that it compares favorably with other methods of segregation analyses. The results of these calculations are shown in Table 4, in which they are compared to the calculations for the Cebu data. For the Cebu data the value of p' is 0.2461 (± 0.0585), a close fit, and for Bougainville the value is 0.2527 ± 0.0452, also a nice fit. Both of these results compare favorably to the values used as an example by Li and Mantel.

TABLE 4. *Summary of calculations by Li and Mantel's[34] method for Cebu and Bougainville Au(0) × Au(0) families.*

	t	j	r	p'	Standard error
Cebu	85	20	36	0.2461	0.0585
Bougainville	124	33	56	0.2527	0.0561

These segregation analyses taken together with the previous Cebu study strongly support the hypothesis that the trait follows simple Mendelian segregation in at least these two populations.

If the distributions in the families were due to simple infection with no genetic effect, then it would be expected that the frequency of Australia antigen in the genetically unrelated spouses of Au(1) individuals would be about the same as the frequency in the genetically related children. There were 33 families in which both parents were tested and at least one was Au(1). There were 27 Au(1) among the 97 offspring, but none of the mates were positive, and this difference is significant ($0.01 > p > 0.001$).

Although there may be some nongenetic explanation for this distribution, as suggested in our previous paper,[12] the close correspondence of the observed to the expected numbers of recessives in two quite different communities (Cebu and Bougainville) adds weight to the validity of the genetic hypothesis.

The data summarized in the introduction indicate that Australia antigen may also behave as an infectious agent. These findings are compatible with the explanation that there is an inherited susceptibility to chronic infection with Australia antigen mediated by the Au^1 gene. In populations where the Au^1 gene is relatively common and where the infectious agent is also common, then all those individuals susceptible to the agent would become infected and the segregation in the families would appear to follow a pattern of autosomal recessive inheritance.

By analogy with other examples of inherited susceptibility to infection, there are probably factors such as age, sex, state of nutrition, etc. which also affect susceptibility and resistance. Under appropriate circumstances an individual without the inherited susceptibility factors could become infected. Most patients in the United States who contract acute viral hepatitis (usually post-transfusion hepatitis) may have the antigen transiently, but it is only rarely chronic, since they are not genetically susceptible to the chronic infection. In these patients, the presence of the antigen is usually associated with evidence of clinical hepatitis and/or highly elevated serum glutamic pyruvate transaminase levels. In individuals with chronic Australia antigen, such as the large numbers of apparently normal individuals in Asia and Oceania, the effect on the liver is much less striking and may be reflected only in minor elevations of serum glutamic pyruvate transaminase, or in no changes at all. A symbiotic accommodation of the organism with the host could have developed in these individuals over the course of many generations, and even though they become infected more readily, they do not develop severe symptoms of the disease. This may represent a protective value of the Au^1 gene and could be associated

with the development of what appears to be a genetic polymorphism in this population.[36]

The data presented here are compatible with the hypothesis that there is an inherited susceptibility in humans to chronic infection with a virus of hepatitis. The susceptibility, in this analysis, appears to be controlled at a single autosomal locus, and individuals homozygous for a recessive gene termed Au^1 are more likely to be chronically infected. The infection is not accompanied by any apparent illness, although people with Australia antigen are probably hepatitis carriers.

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