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Methodology of Genetic Study in the Epidemiology of Leprosy[1]

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I do not intend today to discuss the results obtained thus far in the study currently undertaken by the Leonard Wood Memorial on genetic factors in the epidemiology of leprosy. I would rather explain why we are making this study, and how it is being done, emphasizing some problems of methods, definitions, and sampling, with the hope that these will raise stimulating points for discussion.

The hypothesis underlying this study is that some genetic factors play a role, either in the development of leprosy in an individual or in the type of leprosy a patient will manifest once he is affected by the disease.

Why such a hypothesis?

It appears that the epidemiologic picture of leprosy presents discrepancies that not only cannot be explained fully on the basis of infection alone, but will be reconciled if it is assumed that individuals, or populations, display in some instances an inherent resistance to the disease or specifically to its lepromatous type.

These features are exemplified by the following:

1. The fact that a large proportion of individuals presumably at risk in communities where leprosy is common do not develop the disease, or at least do not develop manifest signs of it: about 95 per cent of the spouses of leprosy patients, husbands or wives, remain unaffected. As shown in Culion, the Philippines, 80 per cent or more of the children do not develop the disease, even if they are born of lepromatous parents and raised in the highly contaminated environment of a leprosarium.

2. The duality of leprosy as a morbid entity, with its two polar types, lepromatous and tuberculoid, and the large variations found in the ratios of these two types in different populations, from 7 per cent lepromatous cases among patients in Africa to 50 per cent or more in Brazil.

3. The persistent lack of lepromin reactivity in certain individuals, even after administration of BCG. If these individuals later develop leprosy, they have more chance to develop the lepromatous type of the disease.

4. The pattern of spread of leprosy in populations in which the disease is introduced for the first time. Examples of leprosy outbreaks, extending over some years, and affecting up to one-third of the population, have been described in some islands of the Pacific, whereas in other islands the disease is reportedly restricted to two or three families over several decades.

Hypotheses other than a genetic one could be put forward; i.e., nongenetic hypotheses might explain some of these peculiarities. One may speculate that immunologic factors, especially cross-immunity with other mycobacterial or nonmycobacterial infections, play a role in the development of acquired resistance to leprosy or to lepromatous leprosy. A large proportion of inapparent infections in the population could explain the low prevalence generally reported in many instances. Variations in the ratio of the two types in different populations may be the result of survey, lepromatous cases being more readily diagnosed. On the other hand, a genetic mechanism controlling resistance to leprosy, or resist-

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ance to lepromatous leprosy, could reconcile many of these discrepant features.

Several methods are available for exploring the role of genetic factors in disease, e.g., study of concordance in identical as compared to fraternal twins, family aggregation, consanguinity, linkage studies, and genetic polymorphism.

In leprosy, the first four methods mentioned have many pitfalls with regard to logistics, sampling, and statistical analysis, which I shall not pause to consider. They could be discussed usefully after this introduction.

The investigation of genetic polymorphism has been selected in the study presently undertaken under sponsorship of the Leonard Wood Memorial. Polymorphism, as many of you know, has been defined by Ford([2]) as the occurrence together, in the habitat, of two or more discontinuous forms of a species, in such proportion that the rarest of them cannot be maintained by mutation only([1]). Examples of polymorphism in men are, among many others, the various blood group systems; some hemoglobin variants, such as HbS, which is responsible for the sickle-cell trait; and several proteins of the serum. Populations are heterogeneous with respect to the frequency of the different allelomorphic genes responsible for these characteristics, and therefore show definite proportions of the resulting phenotypes, e.g., the distribution of the ABO blood group, the prevalence of the sickle cell trait, etc.

In a given population, gene frequencies, and correspondingly phenotype frequencies, may be studied in relationship with some attributes, such as diseases.

For example, Allison([1]) has shown, in West Africa, that the prevalence of sickle cell trait is lower in individuals affected with malaria caused by *Plasmodium falciparum,* than in persons not affected by malaria, hence demonstrating a relationship between a genetic factor, sickle cell trait, and a communicable disease.

The discovery of a statistically significant association between a disease and a known genetic factor, implying that some genetic factors are at work in the etiology of the disease, is the first step. There are two, or even three, further steps, as follows:

The second step is to confirm epidemiologically, by a longitudinal study, and/or even experimentally, observations made in sectional genetic surveys. It is necessary to demonstrate, as has been done in malaria, not only that people with malaria have a lower prevalence of sickle cell trait, but that people with the sickle cell trait, followed for a sufficient period of time, have a lower incidence of malaria.

The third step is to investigate the mechanism by which the genetic factor involved plays a role in susceptibility or resistance to the disease. This is the difficult task for the basic scientist. (Why, for example, does HbS not support *Plasmodium falciparum* as well as HbA?)

A fourth step is to study the interaction, over several generations, between the gene frequencies in the population and the incidence of the disease among the individuals constituting this population. This means building a mathematic model representing the modifications in the gene frequencies brought about by differential mortality or fertility from a disease whose chance of development depends on the genetic constitution, that is to say, to study how the disease exerts a selection in the population.

In leprosy we are concerned at present only with the first step, i.e., to find an association between the disease and some genetic marker. Because of the long incubation period of the disease, the difficulties encountered in basic research, especially the cultivation of *M. leprae,* and the lack of valid mortality or fertility data, the later steps must be deferred.

At present we are investigating eight blood group systems, viz., ABO, MNSs, Rh, (CDEcde), Kidd, Kell, Duffy, and Lutheran; an enzyme G-6-PD (glucose-6-phosphate dehydrogenase); and various proteins of the serum, including transferrins, haptoglobins, Lp (beta-lipo-proteins), and Australia antigen.

Laboratory studies are being performed on specimens drawn from approximately 1400 individuals on Cebu Island, comprised of about equal numbers of healthy controls, lepromatous patients, and tuberculoid patients.

Besides logistic problems, e.g., shipment of specimens in dry ice, and problems of technic—the study involves the cooperation of several scientists in different laboratories—, this investigation has raised interesting questions of sampling, definitions, and classification. I shall mention only a few:

1. Classification of the type of leprosy in nonactive cases on the basis of agreement between clinical investigation at present and past immunologic and bacteriologic records, by two observers.
2. Assurance of random sampling in the study group.
3. Selection of healthy controls, matched for age, and as far as possible also for exposure to leprosy, as well as can be judged from similar conditions of environment. In the present study the controls consist of persons consulting at the Cebu Skin Dispensary for diseases other than leprosy, it being assumed that patients in whom leprosy was excluded are of socio-economic status similar to that of the patients.

Sometimes bias in sampling of the controls is not immediately apparent, and this should be guarded against. For example, blood donors in many instances could constitute a poor sample of controls, universal donors, or persons with rare blood groups, being more likely to attend a blood bank. Similarly, parents, i.e., fathers or mothers who bring their children to well-babies' clinics, constitute a readily available pool of healthy controls for all kinds of studies. Yet it must be realized that, for supply of controls in an investigation dealing with the sickle cell trait, for example, this probably constitutes a highly biased sample, parents with children affected by, or dead from sickle cell anemia, and therefore carriers of the trait, being much more likely to attend such clinics.

These difficulties, plus the many biases, make this type of study fascinating. Proper sampling is of the utmost importance for provision of valid statistical comparisons. Yet biologic investigations, and among them particularly the epidemiologic ones concerned with man, deal with populations of highly diversified individuals.

In order to achieve a valid interpretation of the data, an awareness of the possible imperfections of the selected sample is possibly as important as efforts at securing a good sample.

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DISCUSSION

Dr. Sartwell. The discussion of this paper and of Dr. Blumberg's will be opened by Dr. Bernice Cohen. Dr. Cohen is in the Department of Chronic Diseases at Johns Hopkins and in charge of the human genetics program there.

Dr. Cohen.[1] Since time is limited I thought it best to jot down a few remarks and questions so as to leave the maximum amount of time for Dr. Lechat and Dr. Blumberg and other participants to discuss points I might raise.

It is clear, I think, from the presentations and discussions that the nature of leprosy, its failure to manifest itself in some exposed individuals, its differential manifestation in others, its differential distribution in populations of the world and subgroups of those populations even under similar conditions of exposure and environment, all make this disease a likely candidate for a hereditary component. This is certainly not a new and revolutionary idea. The literature is replete with studies of the genetic aspects of leprosy, dating back to the mid-1800's and even further. But, surprisingly, while

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much has been written on genetic factors in leprosy, careful examination reveals that there has been very little that is conclusive. This I believe has been pointed out already by Dr. Blumberg, and intimated also by Dr. Lechat, who were both looking ahead for answers in their own studies. There isn't time here to evaluate the various past investigations, but I think it is worthwhile to take a few moments to look at these studies and perhaps give a few examples of their contradictory nature. The chief points are illustrated in the accompanying tables.

Table 1. On the left hand side are shown some attack rates of sibs in families where either both or no parents were affected. The data recorded come from Dr. Mohamed Ali ([6]) of Chingleput, India. Where fathers were affected the attack rate was 36 per cent, where mothers were affected it was 43 per cent, where both mother and father were affected it was 35 per cent, and where both parents were free from the disease the rate was 34 per cent. Clearly, there is no difference in the offspring attack rate associated with parental disease in this study.

On the right hand side are some data from Sand and Lie ([8]), which were cited by the same author (Mohamed Ali) ([6]), and here we find that where fathers were affected the attack rate was 7 per cent, where mothers were affected it was 14 per cent, and where both parents were affected it was 26 per cent, which shows a considerable difference. Now, how can we reconcile these findings?

With regard to type of manifestation, according to some investigators the data suggest that relatives tend to suffer from the same form of disease. According to other investigators, however, the secondary cases produced by a particular type of index case within a family were not of the same type in the majority of cases ([6,10]).

Possibly some of these contradictions are due to the use of highly selected samples, and/or the lack of control data where needed. Both are difficulties that permeate most of the family and twin studies in both leprosy and tuberculosis, which Dr. Blumberg used as a comparison disease. And I think it might be appropriate to point out some of the limitations of the past studies here, in order to prevent others also from being led astray.

First of all, I think that the twin studies in leprosy, because of the problem of limited numbers and questionable reliability of zygosity determinations, as well as sampling problems, are not highly informative, as they now stand. Nevertheless, the twin method itself is used in this complex disease, in which there is certainly an environmental component, because in twin studies there is no attempt to pin down any specific mode of inheritance. Similarly, I think that familial aggregation studies examining patterns of familial occurrence, particularly with regard to the type of manifestation of the disease, and also with regard to lepromin reactions (i.e., familial aggregation studies that do not test for established *a priori* expectancies, but look for patterns of family occurrence) are very valuable in elucidating genetic factors in this disease.

TABLE 1. *Attack-rates of sibs in families where either, both, or no parents were affected.*[a]

	(Mohamed Ali, 1965)			(Sand & Lie)		
Parents	No. of families	No. of sibs	Attack rate %	No. of families	No. of children	Attack rate %
Father affected	150	485	36			7
Mother affected	89	252	43			14
Both mother and father affected	26	93	35			26
Both parents unaffected	750	2424	34			
Total				587	2010	

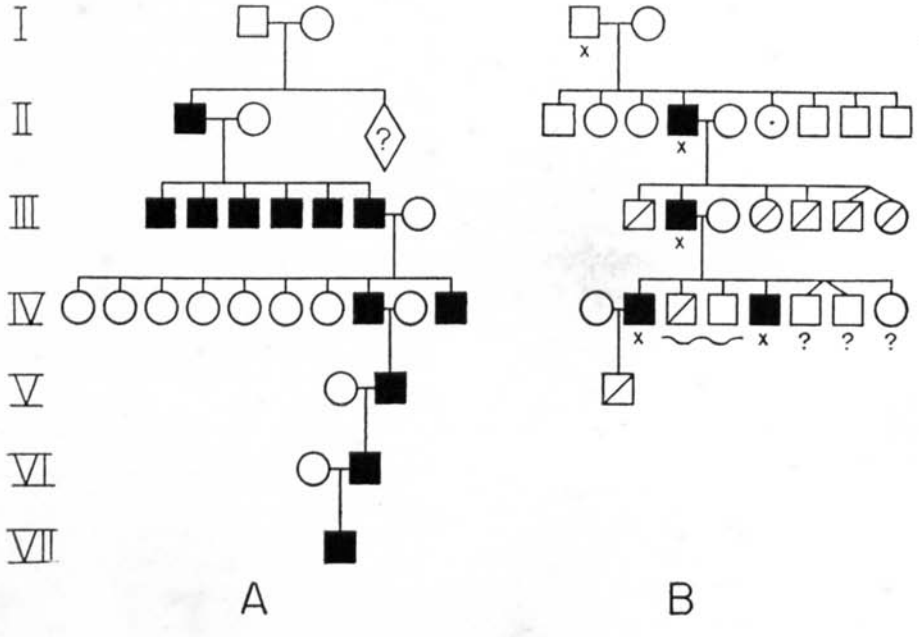
[a]Data from Mohamed Ali.

Now, these types of studies are in contrast to many of the family studies already carried out, which have sought to specify simple modes of inheritance, and which, on this basis and on numerous other grounds, such as sampling and genetic theory, really defy interpretation.

Some investigators, after presenting intelligent criticism of the work of others and the problems involved, have then plunged headlong into some rather unwarranted assumptions concerning the heredity of leprosy, and often have used single pedigrees for analysis of mode of inheritance. Incidentally, the data that Dr. Blumberg presented, i.e., the data in which the mode of inheritance was analyzed, came from a single Acadian pedigree, I believe, and he quite properly questioned its reliability for conclusions regarding the mode of inheritance of leprosy. I would like, therefore, to point out some of the reasons for questioning the reliability of using pedigree data for this purpose, because I think it might be worthwhile to those who are planning future studies on genetic aspects of leprosy.

First, data in the pedigrees that involve many generations usually have been collected retrospectively, and diagnostic accuracy passed down over four generations is usually not very reliable. I might cite an example from another disease, ichthyosis hystrix gravior. Figure 1 illustrates it.

The Lambert pedigree, which is shown on the left side, was published for many years in genetic textbooks as a model of Y-linkage. However, upon reinvestigation based on records and documents, it is no longer considered to be an unequivocal example of Y-linkage ([11]). Now, both pedigree *A* and pedigree *B* represent the same pedigree. In the one indicated above *A* you will see that all males, and no females, are affected. This is exactly what one would expect in simple Y-linkage. After reinvestigation, using records and other documented data, Curt Stern and Penrose in England were able to come up with a corrected



A. After cockayne.

B. After penrose and stern. symbols with an oblique line indicate that these individuals were reported to have been affected. the wavy line in iv indicates that the sequence of iv-3 and iv-4 among themselves is unknown. the zygosity of the twins in iv is unknown. the question marks signify absence of information concerning trait.

Fig. 1. Ichthyosis hystrix, *the Lambert family (from Stern, 1957).*

pedigree which you see is very different from pedigree *A*. Pedigree *B* differs in that not all males were affected, and some females probably were affected. The oblique line drawn through the circles indicates females who were probably affected, and the condition of some of the members of the family indicated by question marks, remains unknown. In any case, it is clear that use of retrospective data, gathered from secondary sources, is not very valuable in determining a mode of inheritance in genetic study.

Secondly, in criticism of some of the past family studies, and as a warning for future studies, I might indicate also that individual single pedigrees or groups of pedigrees collected from the literature are almost useless for determining the mode of inheritance of traits of comparatively high frequency and late onset, since these selected pedigrees are certainly not representative samples of any clear-cut reference population. Often they have come to attention because of multiple cases in the families, or have been selected for recording for some specific, albeit unknown reasons.

Thirdly, as Dr. Blumberg indicated, it is risky to invoke a secondary hypothesis of reduced penetrance for data that do not fit single factor expectancies, since it is not possible to distinguish between a dominant with limited penetrance occasionally skipping a generation, and a recessive trait of appreciable frequency in the population, especially in diseases that are neither very rare nor always present at birth. And this would be the case in leprosy, which is neither very rare in many parts of the world, nor found congenitally.

James Neel ([7]) discussed this problem of the secondary hypothesis in his presidential address to the American Society of Human Genetics a few years ago, bringing out some pitfalls in making genetic inferences.

Thus, the assumption of a single irregularly dominant gene in leprosy, as had been proposed ([9]), does not seem justified on the evidence available, and the suggestion of a multifactorial basis ([10]) is much more reasonable.

Fourthly, and finally, statistical analysis using population methodology assumes that there is a large randomly mating population, and that the conditions of the Hardy Weinberg equilibrium prevail. Therefore, the application of population technics to data derived from a single pedigree or other types of biased samples, is almost meaningless.

Clearly then, a review of past work on the subject suggests that we must look ahead for clarification of the role of genetics in the epidemiology of leprosy, and that this may come from searching beyond hypotheses involving simple single factor inheritance as directly and specifically responsible for susceptibility to leprosy.

Today, we heard from Dr. Lechat and Dr. Blumberg of a study in progress under the sponsorship of the Leonard Wood Memorial in an investigation to determine if there are any associations between leprosy (susceptibility, manifestation, and/or prognosis), and genetic traits. I would like to ask Dr. Lechat and Dr. Blumberg to discuss some of the procedures they are using, as well as some of the problems involved in their study, explaining how they avoid some of the traps others have fallen into.

First, would Dr. Lechat discuss the methods used for his cross-sectional survey of the population, including his sample selection, his control selection, and some of the problems he has encountered, and, if he has time, perhaps a few remarks on age, sex, and the diagnostic criteria of disease.

Second, would Dr. Blumberg discuss some of his proposals for sampling in any family studies he would plan?

And, third, I would like to address an open question to any of the participants with regard to another problem that has concerned me in the course of reviewing the literature. I presume that there might be a great difference of opinion with regard to this problem that has been puzzling me. I would like to know whether any observed genetic association necessarily would be expected to parallel the scheme of disease severity; that is, with lepromatous leprosy necessarily at one end of the scale, and no disease manifestation at the other, and tuberculoid disease in the middle. Is it at all conceivable that persons

with the tuberculoid type of disease might be more markedly different from controls than lepromatous individuals are from controls? Some associations have been reported. It may be that the studies are unreliable and may turn out to be completely negative, but, in any case, these studies have shown rather bizarre patterns and I would like to hear those who are specialists in leprosy discuss this point. It would be very helpful to those geneticists among us who are working on the problem.

I would now like to show you some of the data that have been reported from other investigators.

Table 2 shows some of the results of Hsuen and coworkers ([5]) published in 1963. Hsuen discovered a significant difference in the ABO frequency in leprosy patients and controls. But you will note his finding that in leprosy patients the O to B ratio was 1.75 times the ratio in controls. In lepromatous patients the ratio was only 1.48 to 1, whereas in nonlepromatous patients the ratio was somewhat over 2, so that the nonlepromatous patients showed more marked deviation from the controls than the lepromatous patients. One might criticize this study since the controls are not of the same age or sex distribution as the cases, and leprosy was excluded in the controls only by gross examination. So I do not feel that these results have to be taken at absolute value; I merely want to raise the question as to whether such results could be explained if they were found.

TABLE 2. *Relative incidence of leprosy in "O" group as compared to "B" group in the study series (lepromatous and tuberculoid patients compared to controls).*[a]

Type of leprosy	Relative incidence O : B
Lepromatous patients	1.48 : 1
Nonlepromatous patients	2.06 : 1
TOTAL: Lepromatous and nonlepromatous patients	1.74 : 1

[a]After Hsuen *et al.*, 1963.

Dr. Beiguelman ([1]) also has reported that tuberculoid patients had an excess of O when compared with the nonleprous population, but that the O frequency in lepromatous patients was not significantly different from that in the nonleprous population. He has also found a difference in regard to PTC ([2,3,4]). Again, we might criticize these findings on the basis of his control group. Nevertheless it is interesting that the tuberculoid patients were more different from the controls than the lepromatous patients. So, granting that one cannot accept these conclusions, we note that the findings do raise an interesting question. Can expectation of a gradation of associations, with tuberculoid intermediate between lepromatous and no disease at all, hamper our views, and is this even unrealistic to expect? Perhaps we must think in terms of more complex genetic models with interacting environmental components. On that note I shall close and leave the floor open for the speakers and other discussants.

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Dr. Hart. I would like to refer to the difficulty of carrying out twin studies, e.g., in tuberculosis. Dr. Blumberg quoted the well-known study by F. J. Kallmann and D. Reisner (*American Rev. Tuberc.* **47** (1943) 549-574) who concluded that there was a strong inherited factor in tuberculosis. In 1963, Simonds, working for the Royal College of Physicians Prophit Committee, published a report (Simonds, B. Tuberculosis in Twins. London, Pitman Med. Publications, 1963) on a large study of this nature in England. She stressed the importance of total collection of twin index-cases, which had been a difficulty in the case of Kallmann and Reisner's work. The result showed considerably less evidence of genetic factors, and even that which was indicated was subject to the influence pointed out by Dr. Blumberg of environment in producing more contact in uniovular than binovular twins.

Dr. Sartwell. After consultation with Dr. Binford, who must share the credit here, I must again acknowledge that we have not time for further discussion. Dr. Binford assures me that if the highly important questions raised by Dr. Cohen and in the two papers that preceded hers can be answered by any present, consideration will be given to publication of their views later.

Dr. Binford. I'm afraid we will have to bring this session to a close on account of pressing problems for the rest of the afternoon. I wish we did not have to do this, but in order to go on with the rest of the program we will have to close formal discussion.